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Correlation of tuned aperture computed tomography with conventional computed tomography for evaluation of osseous healing in calvarial defects

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Abstract

Objective—To compare the diagnostic efficacy of iteratively restored tuned aperture computed tomography (TACT) with conventional computed tomography (CT) for evaluation of osseous healing in induced calvarial defects.

Study design—Fifty-six calvarial defects in 14 rabbits received 1 of 4 possible treatments: copolymer membranes with and without bone marrow stromal cells (BMSCs), BMSCs alone, or no treatment (control). Healing was measured after 2, 4, and 8 wks as remaining defect areas measured on TACT and CT images. Histomorphometric analyses were done on the specimens.

Results—Bone formation was minimal to none in control defects and those treated with BMSCs or polymer matrices alone. Healthy bone formation was noted in defects treated with polymers impregnated with BMSCs. Unresolved defect area measurements using TACT and CT of osseous healing showed a high positive correlation.

Conclusions—Potential for TACT to accurately detect osseous healing in surgical defects was demonstrated. High resolution of TACT combined with generation of information in 3D yields comparable performance to CT.

Early osseous or nonosseous healing is difficult to detect using conventional imaging techniques, necessitating the use of advanced imaging modalities.¹⁻⁴ Besides localization of healing in 3 dimensions (3D), the use of conventional 2-dimensional (2D) imaging techniques fails to provide definitive spatial information regarding the nature of the process.¹⁻⁴ Detection and quantification of such changes are central to studies investigating the use of different treatment modalities that are evaluated to test for favorable outcomes after surgery in as short a period of time as possible to hasten healing and ensure complete closure of the defect. Most such studies are carried out in animal models in tightly controlled experimental conditions as a preliminary step,¹⁻⁴ followed by pilot studies in humans. Radiographic monitoring assumes significance in that it provides a means for evaluating the nature of osseous healing if any, noninvasively. However, conventional radiographic techniques are fraught with challenges that prevent a meaningful assessment of osseous healing owing to the inability to evaluate the region of interest (ROI) in 3 dimensions and in small increments without superimposition of overlying anatomy.¹⁻⁴ Qualitative and quantitative radiographic techniques exist to address this problem, with different levels of complexity.⁴

One such technique is tuned aperture computed tomography (TACT), which uses multiple 2D projections acquired with task-specific projection geometry to generate a 3D volume to facilitate examination of the region of interest in a noninvasive manner.¹⁻⁷ This study was designed in part to evaluate the diagnostic efficacy of TACT in direct comparison with conventional computed tomography (CT) for evaluation of osseous dynamics in a well controlled experiment in rabbit calvaria. Known limitations of CT for such applications include higher dose, cost, access in research facilities, and potential for motion artifact. Use of a simpler image generation modality such as TACT is therefore justified. Besides, TACT could also be used in human studies, unlike other advanced high-resolution modalities such as microCT, with relative ease. Advantages of TACT have been discussed in great detail in previous studies.¹⁻⁷

This study was a feasibility project to determine if osseous healing in calvarial defects could be detected accurately using TACT and to compare the results with those obtained from CT studies. The remaining defect area at distinct points in time was measured for each induced defect using both imaging modalities. Ground truth was assessed using histomorphometric analyses within the regions of interest. Also of interest was evaluation of the effect of treatment modalities on the extent of healing as detected by TACT and CT.

MATERIAL AND METHODS

Fifteen rabbits were used in the study. Three groups of 5 rabbits each were followed for different periods of time as outlined below. The following treatment modalities were used in the study: 1) bone marrow stromal cells (BMSCs) alone; 2) polymer without BMSCs; 3) polymer with autologous BMSCs; and 4) no treatment. Thus, a total of 60 sites were evaluated, with each rabbit receiving all 4 treatment modalities on the calvarium (Table I). BMSCs were obtained from the long bones of inbred New Zealand white rabbits (Myrtle Rabbitry, Tompkins Station, TN), using protocols approved by the Institutional Animal Care and Use Committee, University of Pittsburgh. BMSCs were harvested from long bones that

were excised aseptically after killing similar rabbits (Fig. 1). Polylactide/polyglycolide film polymer (PLA/PGA) matrices were obtained from Aatrix Laboratories, Fort Collins, CO. BMSCs were grown on PLA/PGA matrix films.

Rabbits were anesthetized by an intramuscular injection of 80% Ketajet and 20% Xylajet (0.1 mL/100 g body weight), were shaved at the calvarial region, and cleaned with antiseptic. Fifty-six defects were induced in rabbit calvaria using an 8-mm-diameter trephine by a single investigator (Fig. 2). All defects were placed 6 mm apart to avoid crossover of cells. The dura mater was left intact. Prior to implantation, the defects were flushed with saline to remove bone debris. Calvarial defects in each rabbit received one of the treatment modalities mentioned earlier. Control defects were not filled, but sutured after removal of the bone and periosteum. Bone formation in defects was tested for a period of 2, 4, and 8 wks. This period was deemed sufficient to evaluate bone healing based on studies done before.² In order to avoid the possible effect of the location of the defect on treatment outcome, treatment modalities were rotated among defect sites in different animals in no particular order. However, an equal distribution of treatment modalities among specimens was ensured.

Evaluation of bone formation in the defects was carried out using TACT and CT imaging. Groups of animals were killed after 2, 4, and 8 weeks, and calvaria with retained soft tissue were retrieved for imaging. The specimens were placed in positioning jigs for image acquisition, and the x-ray source of a CommCAT unit (Imaging Sciences International, Hatfield, PA) was used to acquire the basis images. This was done to achieve reproducible projection geometry for the limited number of specimens imaged so that projection-related variables were controlled to some extent. Direct digital images were generated using a #2 size CMOS-based Schick sensor (Schick Technologies, Long Island City, NY), which was placed along the interior surface of the calvaria. A 1-mm-diameter fiducial marker (X-Spot; Beekley Corporation, Bristol, CT) was placed in a central location between the calvarial defects to facilitate image reconstruction. A controlled projection geometry was employed by moving the x-ray source to different loci in space to generate images. An angular disparity of up to 10 degrees to either side of the orthogonal projection geometry was employed for a total of 16 projections. The exposure parameters used were 65 kVp, 8 mA, and 350 ms, with a source-to-object distance of 45 cm. The basis images were imported into the TACT workbench (Wake Forest University, Winston-Salem, NC). TACT slices were generated and subjected to iterative restoration.¹⁻³ Slices generated through comparable planes were evaluated across all defects. The images were assigned random identifiers and presented in no particular sequence to 2 observers who had previous experience with interpreting TACT images but had not participated in the surgical phase of the treatment nor had access to information on treatment modalities used in each defect. The observers participated in a training session first. Images were presented, 1 defect at a time in no particular order for the study sessions. The observers were asked to measure the largest unresolved defect area within the volume for each induced defect, the margins of which were discernible on all radiographs. The slices were examined in an interactive mode by the observer and the area of the largest defect detected within the volume of interest was recorded. The readings were repeated twice in individual sessions separated by at least 2 weeks for each defect and the mean reading recorded. The readings were very similar, with little difference between the 2 sets. All images within each of the imaging modalities

evaluated were histogram equalized with respect to a reference image. An ROI within a site was selected and referenced so that it could be applied to homologous sites across all specimens.

Calvaria were also examined using CT in the coronal plane in a GE HiSpeed Advantage Scanner (FOV = 4 cm; mA = 120–150; kV = 120) at slice thicknesses of 1 mm (contiguous). Three-dimensional reconstructions of each calvarium were generated also. The inner boundaries of the calvarial defects were manually traced from the external cortical surface of the reconstructions, and the defect area was calculated for each defect using Allegro Software (ISG Technologies, Atlanta, GA) on a Sun workstation. CT area measurements were carried out by the same investigators in a blinded fashion (Fig. 3).

Area measurements obtained using TACT and CT were compared. Mean defect areas were calculated and compared among groups using a 2-way (group by postoperative time) analysis of variance (ANOVA) with means analysis. Intergroup differences were assessed using the least significant differences multiple comparison test (SPSS 1997; SPSS, Chicago, IL). Differences were considered significant if $P < .05$.

Histologic analysis served as ground truth. Groups of rabbits (4–5 in each group) were killed 2, 4, and 8 weeks after surgery for this purpose. The formalin-fixed calvaria were then decalcified in 10% formic acid, trimmed, and embedded in paraffin, and the defect area was cross-sectioned, stained with hematoxylin and eosin, and examined microscopically using ImagePro software (Media Cybernetics, Silver Spring, MD). The defect was cut at the center, and 10 sections, 200 μ m apart, from the center of each side of the defect were examined. The total area of defect and areas of bone formation in defects were traced and calculated. The ratio of bone to total defect was calculated on a scale of 0 to 100, where 0 represented no bone formation and 100 represented complete filling of the defect by bone. One calvarium (in the 2-wk group) was lost to sectioning and therefore no data were available.

RESULTS

Changes in mean defect area by group and postoperative time were measured for all images. In CT and TACT images, it was noted that at 4 wks postoperatively, the defects in the BMSC-only (Fig. 5) and control groups (Fig. 6) were reduced by approximately 35%, whereas the defects in 2 groups with membranes were reduced by approximately 83%. At 8 wks postoperatively, the defects in the control and BMSC-only groups were reduced by approximately 75%, the defects in the membrane-only group were reduced by approximately 65%, and the defects in the BMSC and membrane group were reduced by 85%. A 2-way analysis of variance revealed a significant ($P < .05$) group by postoperative time interaction. The defects with membranes changed significantly ($P < .05$) over time compared to those with no membranes. Defects treated with polymer matrix and BMSCs demonstrated maximal bone formation and defect resolution (Fig. 7). TACT and CT readings showed positive correlation. Pearson's correlation coefficient for area measurement of remnant defects using TACT and CT was 0.80.

The defects filled with only polymer-matrix films (Fig. 4) showed formation of dense connective tissue extruding from the defects with inflammatory cell infiltration. Histomorphometry confirmed the extremely dense nature of fibrous connective tissue, the presence of matrix remnants, as well as the obvious inflammatory reaction. These defects showed partial bridging of the defect at the end of 4 wks but relatively less bone formation in TACT (Fig. 4) and CT images. Any new bone formation that was noted histologically extended from the margins of the defect toward the center as an extension of the peripheral lamellar bone. It is noteworthy in all of these situations that bone formation was relatively less in the absence of BMSCs. Remnant defect sizes increased at the end of 8 wks.

Histomorphology of defects filled with BMSC suspension also exhibited a dense fibrous plug protruding from the defect. Figure 5 shows the TACT image. Histologically, these defects showed relatively less ossification compared with those treated with BMSCs and polymer combination. Four defects exhibited in-growth of bone from the edges of the defect close to the dura. These defects also exhibited extensive connective tissue formation and absence of foreign body responses.

Unfilled (control) defects showed formation of connective tissue extruding from the defect by 4 and 8 wks. Resolution of defects was poor. Figure 6 shows the TACT image of a control site. The histologic analysis of unfilled sites in all animals showed very little, if any, bone formation in the defects over the period of the study. CT revealed that the defect was devoid of cancellous bone at the end of the study.

However, in defects with polymer matrix and BMSC suspension combination (Fig. 7), histomorphometry revealed that $72\%–86\% \pm 6\%$ of the area was covered with well vascularized ossified tissue after 4 wks. Fibrous connective tissue was not apparent in these defects. The defects were completely bridged on the external cortical surface. Both TACT and CT images revealed osseous resolution of the defects. By 8 wks, the defects were completely resolved radiographically.

DISCUSSION

This well controlled study serves to illustrate the utility of TACT in assessment of healing of osseous defects treated with different types of grafts. Artificial lesions were induced to control for variables introduced by naturally occurring lesions that would be difficult to find and standardize based on etiology. Previous studies have indicated that critical size defects healed in a period of 12 wks using the polymer membranes employed in this study, whereas other studies have indicated failure of resolution of such defects over a longer period of time.^{8,9} Accurate noninvasive determination of defect resolution is a challenging task, for which advanced imaging modalities are typically used.^{10–14} This study served to demonstrate that TACT could be used for this purpose with relative ease.

This study also confirmed that the presence of a scaffold that was biocompatible and susceptible to less inflammatory cell response and impregnated with BMSC suspension assisted in rapid and nearly complete healing of relatively large calvarial defects with well vascularized osseous tissue. Control defects and those with cells or polymer matrix alone, on

the other hand, demonstrated little to no osseous tissue formation although defect resolution was achieved with fibrous connective tissue plugs and mild to moderate inflammatory cell infiltration. Other interesting changes were noted in defects with the use of PLA/PGA matrix alone. Defect resolution was significantly faster in these defects than in sites treated with BMSCs only or control sites. The initial response was similar to those seen in sites treated with the BMSC-polymer combination. However, net bone formation as measured histomorphologically was higher in the latter. It was also noted that healing in sites treated with polymers revealed at the end of 8 wks that the net defect sizes increased from 4 wks. This may be explained by possible remodeling and resolution of the rapid fibrous healing response noted at 4 wks, as the biocompatible PLA/PGA membrane eventually was resorbed. More studies are in order to confirm these changes.

TACT seems to be capable of reliably capturing tissue changes that indicate osseous healing in vivo, which was confirmed histologically. Observed changes on TACT and CT images over the period of the study therefore were not the result of system nonlinearities. The ease with which TACT images were captured and reconstructed to be examined in 3D and the accuracy of measurements as supported by histomorphometry indicate that TACT may be the imaging modality of choice for these types of studies. The high resolution of intraoral sensor used in the study coupled with the availability of 3D information clearly puts TACT ahead of CT theoretically, because CT suffers from resolution limits imposed by the system, primarily along the z-axis. Recently, cone-beam CT (CBCT) using isometric voxels has been introduced in head and neck imaging,¹⁵⁻²⁴ but no studies are available to date comparing it with TACT. The resolution offered by all of the CBCT modalities is inferior to that of TACT; however, image acquisition and reconstruction are much faster and automated, as is the capability to do multiplanar reconstruction using distortion-free images. Significant advantages of volumetric imaging using CBCT include extremely low dose of radiation per study, absence of artifacts that are known to occur with conventional medical-grade CT (such as starburst pattern commonly seen with the presence of high attenuation objects such as amalgam or full coverage restorations in the oral cavity), and low probability of occurrence of motion artifact owing to the relatively short time of exposure.¹⁵⁻²⁴ However, high-resolution imaging tasks may still continue to require the use of imaging algorithms like TACT.

In this study, we pooled data from sites that used polymer matrices alone although different types of matrices were used. The composition was similar but the proportion of different constituents was varied to see if any difference in healing would be noted, including the extent of inflammatory response evoked. However, the sample size being small, it was decided that this effect would not be amenable to statistical testing based on polymer matrix composition. The basic idea was to study the effect of presence of a scaffold on healing with and without BMSCs. Sites with such scaffolding resulted in a more efficient resolution of the osseous defect, as seen in histomorphometry; however, BMSCs were needed to sustain healing and defect closure. More studies are therefore needed to determine the most optimal composition of polymer matrix to achieve desirable closure of the defect even when used without BMSCs.

TACT can thus be used to monitor osseous healing in humans. Patients with destructive lesions resulting in cortical thinning and perforation can undergo TACT imaging to evaluate the extent of the defect. Similarly, healing of surgical defects in craniofacial surgery using tissue-engineered bioresorbable grafts can be monitored. Applications in endodontic and periodontal surgery as well as craniofacial surgery in patients with developmental clefts and defects exist. Surgical procedures involving excision of space-occupying lesions, biopsies, repair of developmental clefts, procedures requiring craniotomy that are routinely done in neurosurgery, and interventional vascular surgery for intravascular lesions located in the cranial cavity will benefit from the use of tissue-engineered grafts to close critical size defects. Monitoring of healing of such defects, if called for, using high-resolution 3D imaging may be done using TACT. TACT seems to have the capability to reliably quantify osseous healing. However, the present study was done in a tightly controlled manner, and extrapolation to the clinical scenario needs to be made with caution for the specific diagnostic task. More studies may be in order using biocompatible resorbable polymers that produce less inflammatory response while stimulating formation of vascularized bone in relatively short periods of time in similar defects using larger sample sizes with limited treatment modalities.

In conclusion, this study indicates that TACT may be an alternative noninvasive imaging modality for detection of osseous changes in healing surgical defects in the cranium if high-resolution imaging is needed or in the absence of access to other advanced imaging modalities.

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Fig. 1.
Bone marrow stem cells growing on the polyurethane scaffolds prior to implantation.

Fig. 2.
Surgical placement of BMSC-impregnated polyurethane scaffolds in rabbit calvarial defects.

Fig. 3.

CT image showing defects that underwent different treatments. Note that complete osseous healing was noted in the defect treated with polymer matrix and bone marrow cells (BMSCs) in 8 wks.

Fig. 4.
Cropped TACT slice showing defect filled with polymer matrix alone after 8 wks.

Fig. 5.
TACT slice showing defect filled with bone marrow cells alone after 8 wks.

Fig. 6.
TACT image showing control defect after 8 wks.

Fig. 7.
TACT image showing defect treated with polymer matrix and bone marrow cells. Note resolution of defect in 8 wks.

Table I

Distribution of lesions by group, time after surgery, and treatment modality

Group	Time after surgery, wks	Treatment modality (n)
1	2	Control (5) BMSC-polymer matrix (5) Bone marrow cells (5) Polymer matrix (5)
2	4	Control (5) BMSC-polymer matrix (5) Bone marrow cells (5) Polymer matrix (5)
3	8	Control (5) BMSC-polymer matrix (5) Bone marrow cells (5) Polymer matrix (5)