RESEARCH ARTICLE

Contrast-enhanced microCT (EPIC- μ CT) *ex vivo* applied to the mouse and human jaw joint

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Objectives: The temporomandibular joint (TMJ) is susceptible to the development of osteoarthritis (OA). More detailed knowledge of its development is essential to improve our insight into TMJ-OA. It is imperative to have a standardized reliable three-dimensional (3D) imaging method that allows for detailed assessment of both bone and cartilage in healthy and diseased joints. We aimed to determine the applicability of a contrast-enhanced microCT (μ CT) technique for *ex vivo* research of mouse and human TMJs.

Methods: Equilibrium partitioning of an ionic contrast agent *via* μ CT (EPIC- μ CT) was previously applied for cartilage assessment in the knee joint. The method was *ex vivo*, applied to the mouse TMJ and adapted for the human TMJ.

Results: EPIC- μ CT (30-min immersion time) was applied to mouse mandibular condyles, and 3D imaging revealed an average cartilage thickness of $110 \pm 16 \mu\text{m}$. These measurements *via* EPIC- μ CT were similar to the histomorphometric measures ($113 \pm 19 \mu\text{m}$). For human healthy OA-affected TMJ samples, the protocol was adjusted to an immersion time of 1 h. 3D imaging revealed a significant thicker cartilage layer in joints with early signs of OA compared with healthy joints (414.2 ± 122.6 and $239.7 \pm 50.5 \mu\text{m}$, respectively). A subsequent significant thinner layer was found in human joints with late signs of OA ($197.4 \pm 159.7 \mu\text{m}$).

Conclusions: The EPIC- μ CT technique is effective for the *ex vivo* assessment of 3D cartilage morphology in the mouse as well as human TMJ and allows bone–cartilage interaction research in TMJ-OA.

Dentomaxillofacial Radiology (2014) **43**, 20130098. doi: [10.1259/dmfr.20130098](https://doi.org/10.1259/dmfr.20130098)

Cite this article as: Renders GAP, Mulder L, Lin AS, Langenbach GEJ, Koolstra JH, Guldberg RE, et al. Contrast-enhanced microCT (EPIC- μ CT) *ex vivo* applied to the mouse and human jaw joint. *Dentomaxillofac Radiol* 2014; **43**: 20130098.

Keywords: temporomandibular joint; Hexabrix; articular cartilage; osteoarthritis

Introduction

Osteoarthritis (OA) is a destructive and progressive joint disease with a large impact on joint function. One of the major characteristics of OA is the progressive damage of the articular cartilage layer. The initiating events are still not fully understood, yet the involvement

of the underlying (subchondral) bone tissue in the process is gaining support. A significant role of the subchondral bone (SCB) is suspected because it acts as an integrated functional unit with the cartilage layer.^{1–5} Like all other synovial joints, the temporomandibular joint (TMJ) is susceptible to the development of OA.^{6,7} OA in the TMJ (TMJ-OA) is likely caused by a disturbed biomechanical environment owing to a history of joint overload.^{6,8} The expected bone changes (*e.g.* sclerosis and SCB plate thickening) may negatively affect the biomechanical

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This project was funded by the Dutch Organization for Scientific Research (NWO grant number: 021.001.050).

Received 7 March 2013; revised 15 October 2013; accepted 17 December 2013

environment of the articular cartilage and cause progressive cartilage degradation and/or damage.^{9–13}

Presently, there is a gap in our understanding about the morphological bone–cartilage interaction in the aetiology of TMJ-OA. More detailed knowledge about TMJ-OA initiation and development is essential to improve our insight into this disease. It is thereby imperative to have a standardized, reliable and, preferably, three-dimensional (3D) imaging method that allows detailed assessment of both bone and cartilage properties in this specific joint in the healthy and diseased situation.

For TMJ-OA research, the normally used histological and biochemical evaluation techniques are incapable of describing the 3D spatial distribution of tissue constituents.¹⁴ Currently available MRI techniques, including delayed gadolinium-enhanced MRI of cartilage, can produce 3D, non-destructive measurements of cartilage *in vivo* in clinical applications in the human knee joint.^{15–17} However, even the most recent MRI systems do not have sufficient resolution to detect local changes in the thin cartilage layers found in the TMJ of humans and small animals.^{18,19}

Conventional microCT (μ CT) can provide fast high-resolution 3D imaging of bone tissue for qualitative and quantitative assessment in the human and the small animal TMJ.^{20–24} Bone abnormalities related to TMJ-OA, like erosion, sclerosis and osteophytosis can thereby be detected and quantified.^{10,23,25,26} Until a couple of years ago, cartilage measurements with conventional μ CT were not feasible owing to too low X-ray attenuations of the soft tissues. However, a novel imaging technique was successfully introduced and applied to the rat knee joint that measured the equilibrium partitioning of an ionic contrast agent *via* μ CT (EPIC- μ CT).^{27,28} With this method, the X-ray attenuation of cartilage is enhanced by treatment of the samples with a contrast-enhancing ionic fluid, and this allowed for qualification and quantification of cartilage morphology and its sulphated glycosaminoglycan (sGAG) composition in small animals.^{28–30}

The TMJ has unique properties compared with other articular joints, like the knee, because it is made of fibrocartilage and acts both as an articular joint cartilage and as a site for endochondral ossification.⁸ The EPIC- μ CT technique has, thus far, predominantly been applied to knee joints.^{28,29,31–36} The dimension of the mouse TMJ is smaller than, for instance, the rat knee joint (width, 0.5 and 4 mm, respectively). Thus, with respect to sample size, a small animal knee joint protocol²⁸ can probably be easily applied to the mouse TMJ to obtain a reliable 3D imaging technique for TMJ-OA research. By contrast, the dimension of the human TMJ is larger (width, 15–20 mm), which might indicate a need for adjustment of the protocol.³⁷ Furthermore, the TMJ predominantly contains fibrocartilage,^{8,38} and the extracellular matrix is composed of less negatively charged sGAGs and more collagen type I fibres compared with hyaline cartilage.³⁹ This can result in a different so-called fixed charged density (FCD) of the cartilage layer in the

jaw joint than in the knee joint. The EPIC- μ CT technique is based on this FCD (as described in more detail in the Methods and materials section), and therefore, the required immersion time for the larger human TMJ is likely to be different.

We hypothesized that it is possible to use the EPIC- μ CT technique to make visualization of the thin articular cartilage layer in the TMJ feasible. The aim of this study was to determine the applicability of EPIC- μ CT for research in both small animal and human TMJs. We assessed the *ex vivo* ability of the EPIC- μ CT technique to provide quantitative 3D morphology of mouse and human TMJ cartilage layers.

Methods and materials

Sample collection

From five 3-month-old healthy female C57BL/6J mice (Harlan, Horst, Netherlands), mandibular condyles were harvested and stored in a 4% phosphate-buffered formalin solution (pH, 7.2; 4 °C). Permission for the use of this material was obtained from the Animal Welfare Committee of the VU University Amsterdam (Netherlands).

A collection of human jaw joints was obtained from embalmed cadavers (fixation fluid: 2.4% formaldehyde, 33% ethanol, 8% glycerol and 0.33% phenol during 1 h). The mandibular condyles were harvested and stored in 4% formalin solution (pH, 7.2; 4 °C). These condylar samples were macroscopically examined and classified as healthy, OA-early stage or OA-late stage (three groups; $n = 5$ per group). The classification was adapted from the principles of Byers *et al*⁴⁰ as follows: Healthy—no visible changes in the cartilage of the type described below; OA-early—early or advanced fibrillation of the articular cartilage or local reduction; and OA-late—total loss of the articular cartilage with bone exposure, or osteophytosis of articular cartilage. The use of human samples conformed to a written protocol that was reviewed and approved by the Department of Anatomy and Embryology of the Academic Medical Center of the University of Amsterdam, Amsterdam, Netherlands.

MicroCT scanning

A μ CT 40 system (Scanco Medical AG, Brüttisellen, Switzerland) was used to obtain and analyse 3D reconstructions of segmented volumes of bone and cartilage. The mouse samples were scanned in air with the inferior side facing downwards. After the immersion in the contrast fluid (contrast-enhancement treatment), the samples were gently dabbed dry. To maintain humidity, a reservoir on the bottom of the sealed specimen holder (diameter: 16 mm) contained a standardized amount of 4 ml phosphate-buffered saline (PBS). The samples were imaged in air using 8- μ isotropic voxels at 55 kVp, 145 μ m and 250 ms integration time. A complete scan required approximately 116 min (397 slices). After scanning, the samples were immersed in PBS for desorption to allow histological processing.

Figure 1 (a, b) MicroCT (μ CT) sagittal cross-section of a condylar sample before and after contrast enhancement. The articular cartilage (AC) showed higher attenuation owing to the immersion of the contrast medium and allowed segmentation. (c) Selection of the regions of interest (scanning-medium = black; articular cartilage = grey; and subchondral bone plate = white) and (d) an example of the produced attenuation-histogram with the relative occurrence of the corresponding voxels. EPIC- μ CT, equilibrium partitioning of an ionic contrast agent *via* μ CT.

The human samples were scanned in air with the lateral condylar facing downwards in a sealed specimen holder (diameter: 20 mm) containing 4 ml PBS. After the immersion in the contrast fluid (contrast-enhancement treatment), the samples were gently dabbed dry. The samples were imaged in air using 18- μ isotropic voxels at 70 kVp, 114 μ m and 250 ms integration time. A complete scan required approximately 36 min (150 slices).

Contrast-enhancement treatment

The EPIC- μ CT technique is based on the principle that diffusion through the cartilage matrix, differs with variable proportion of the local constituents. The cartilage matrix contains collagen fibres and proteoglycans. Negatively charged sGAGs are attached to the proteoglycan backbone, resulting in the negative FCD. A negatively charged radio-opaque contrast agent will be preferably excluded from regions with higher negative FCD associated with higher concentrations of sGAGs.²⁷ Thus, the distribution of the ionic contrast agent (X-ray attenuation in μ CT) is inversely related to the density of the negatively charged sGAGs. The clinically available CT contrast agent Hexabrix® 320 (Guerbet Nederland BV, Gorinchem, Netherlands) was applied, containing the negatively charged hexaiodinated dimer ioxaglate.

The contrast-enhancement protocol for the mouse samples was similar to the EPIC- μ CT protocol described by Xie *et al.*²⁹ immersion for 30 min in 40/60% Hexabrix/PBS solution (37 °C). We expected differences in optimal immersion time for the human samples

owing to the larger dimensions of the human TMJ. Therefore, we decided to test the 30-min 40/60 Hexabrix/PBS protocol and adapt the immersion time if needed.

All human samples were scanned prior to immersion and then at timed intervals of immersion, as described below (Figure 1a,b). The process was repeated for cumulative immersion times up to 20 h to identify the time required to reach equilibrium.

Histological staining for validation purposes (mouse samples)

Following μ CT scanning, the five condyles of five different mice were decalcified in 2.5% formic acid (pH, 4.2; 10 days), paraffin embedded and 7 μ m frontal sections were cut and stained with Alcian blue. For each condylar sample, three sections were used for cartilage thickness analysis. Digital images of each section were captured at a 1392 $\times$ 1040 pixel screen ($\times$ 10 magnification; Leica Qwin Pro, Leica Microsystems, Wetzlar, Germany, Software Leica Microsystems Image Solutions, Rijswijk, Netherlands). Using Leica software, the cartilage thickness was defined as the average value of up to 20 manual thickness measurements at regular intervals perpendicular to the superficial cartilage surface. The histology sections were compared with the corresponding 3D spatial images generated by EPIC- μ CT.

Attenuation-histogram analysis (human samples)

The human samples were used for attenuation-histogram analysis to determine the appropriate immersion time

for reproducible cartilage contrast enhancement. For each scan, 150 images were reconstructed. In the sagittal plane, the images were manually contoured every 10 slices to isolate a region of interest containing the SCB plate (white), cartilage (grey) and the scanning medium (air = black; Figure 1c). Care was taken not to include bone marrow spaces and joint capsule or lateral pterygoid muscle remnants in the region of interests. The manufacturer's software was used to contour the intervening slices and producing a VOI (volume of interest: 150 slices approximately 2.7 mm). Histograms from the attenuation values were obtained to analyse the three peaks corresponding to bone, contrast-enhanced cartilage and scanning-medium voxels (Figure 1d). Per sample, the average attenuation for the cartilage tissue (grey peak) for all three volume of interests was determined and averaged. This average value was directly compared per group between all cumulative immersion times.

Cartilage segmentation procedure

Contour lines surrounding bone, articular cartilage and scanning medium were manually drawn. Care was taken not to include any bone marrow space or soft tissue remnants. To measure cartilage thickness, the cartilage was segmented by choosing global upper and lower thresholds. For mouse samples, these thresholds were 70 and 170 (expressed in threshold units), respectively, and for human samples, 70 and 220, respectively. Thus, in segmented images, every voxel with a linear attenuation within these threshold ranges (assumably representing cartilage tissue) kept their original grey value and voxels below or above these thresholds (representing scanning medium or bone) and were made transparent. Standard gauss filter parameters of sigma = 1.2 and support = 2 were applied. The 3D morphology of the cartilage layer was visualized and quantified in terms of average cartilage thickness (micrometers) using direct distance transformation algorithms.⁴¹ The thickness measurements were obtained for the entire cartilage layer.

Statistical analysis

All data sets were tested for normality using the Kolmogorov–Smirnov normality test (outcome: data normally distributed). Mouse cartilage thickness measurements *via* the EPIC- μ CT technique and histology were compared *via* paired *t*-test, and the relationship and agreement between these two methods were examined separately *via* linear regression analysis (Pearson) and Bland–Altman analysis. For the human samples, ANOVA for repeated measures (Tukey's multiple comparison test) was used to test for differences between the different immersion times within the groups, and one-way ANOVA was used to test for differences between the groups per time point (healthy, OA-early and OA-late). An unpaired *t*-test with Welch correction was used to test the differences in cartilage thickness between the three groups and to determine if the variance within each group

Figure 2 (a) A strong linear relationship between measurements of cartilage thickness obtained by equilibrium partitioning of an ionic contrast agent *via* microCT (EPIC- μ CT) and histology. (b) The differences (histology—EPIC- μ CT) *vs* average cartilage thickness measured by histology and EPIC- μ CT with 95% limits of agreement. *N* = 12 comparisons between histomorphometric measurement and the corresponding μ CT section. SD, standard deviation.

was significantly different. All data were expressed as mean $\pm$ standard deviation. All statistical analyses were performed by SPSS® v. 16.0.2 (SPSS Inc., Chicago, IL) and GraphPad Prism® 5 (GraphPad Software, San Diego, CA). A *p*-value of <0.05 was considered statistically significant.

Results

Histological staining allowed two-dimensional histomorphometric measurements of the cartilage thickness in mouse samples. Cartilage thickness determined *via* histology was not significantly different from measurements provided *via* EPIC- μ CT (paired *t*-test: *p* = 0.220; 95% confidence interval = -2.24 to 8.84). The average thickness of the articular cartilage was 113 ± 19 and 110 ± 16 μ m, as assessed by histology and EPIC- μ CT, respectively. Linear regression analysis of thickness measurements from EPIC- μ CT and histology revealed

Figure 3 Equilibrium partitioning of an ionic contrast agent *via* microCT (EPIC- μ CT) technique applied *ex vivo* to the mouse jaw joint. (a, b) In the μ CT image, a frontal cross-section of the left jaw joint is shown and its three-dimensional reconstruction produced with conventional μ CT. (c) With the EPIC- μ CT technique, the visibility of the articular cartilage is enhanced. A dual-threshold procedure allowed segmentation of the cartilage. (d) The cartilage thickness measured with EPIC- μ CT was compared with histological sections (Alcian blue staining). (e) A thickness map of the cartilage layer (inferior and sagittal view) could be generated with a direct distance transformation algorithm and presented as a pseudocolour scaled image (*i.e.* blue to red; increasing cartilage thickness).

a strong linear relationship ($r^2 = 0.80$, $p < 0.0001$; [Figure 2a](#)). Bland–Altman analysis demonstrated good agreement between these two methods for thickness measurements, with an average thickness difference of $3 \pm 9 \mu\text{m}$ and 95% limits of agreement: $14\text{--}20 \mu\text{m}$ ([Figure 2b](#)). Thus, the two methods agree within

approximately $15\text{--}20 \mu\text{m}$. In the mouse jaw joint, 3D reconstructions of the bone and cartilage components were successfully produced *via* a similar dual-threshold procedure ([Figure 3](#)).

For the healthy human samples, the average cartilage attenuation increased with immersion time from 15 min

Figure 4 The average cartilage attenuation in the three groups as function of immersion time ($n = 5$ samples per group). A significant increase in cartilage attenuation was seen from 0 to 30 min. After 60 min, there were no significant changes seen in any of the groups (values are mean $\pm$ standard deviation). OA, osteoarthritis.

to 1 h. The increase up to 30 min was found to be significant ($p < 0.001$; Figure 4, black bars). We determined similar patterns with cumulative immersion times in the OA-early and OA-late groups (Figure 4, white and striped bars, respectively: $p < 0.001$). Thus, no significant differences were found between the three groups. For the OA-late samples, after a 3-h immersion period, the attenuation values of the cartilage approached the bone attenuation. Therefore, reliable segmentation of the cartilage and bone peaks became unfeasible. For this reason, the results after 3 h were omitted for the OA-late group. To evaluate the method's reproducibility, the complete procedure was repeated twice with a healthy condylar sample. Comparison of the repeated measures

showed no significant differences between the average cartilage attenuations (paired t -test: $p = 0.157$). Based on these results, immersion for 1 h in 40/60% Hexabrix/PBS (37 °C) was selected as a standard protocol for human samples.

Using the adapted EPIC- μ CT technique, 3D reconstruction of the bone components (Figure 5a) as well as the articular layer of the human jaw joint was feasible (Figure 5b,c). The 3D morphology of the cartilage layer could be visualized and quantified in terms of average and local cartilage thickness (Figure 5d). Average cartilage thickness values determined in the human mandibular condyle were 240 ± 51 , 414 ± 123 and 197 ± 160 μ m, for healthy, OA-early and OA-late samples, respectively.

Figure 5 Equilibrium partitioning of an ionic contrast agent *via* microCT (EPIC- μ CT) technique applied *ex vivo* to the human jaw joint. (a) In the μ CT image, a sagittal cross-section of a right human jaw joint and its three-dimensional (3D) reconstruction produced with conventional μ CT are shown. (b) With the EPIC- μ CT technique, the visibility of the articular cartilage is enhanced. (c) A dual-threshold procedure allows for 3D reconstruction of both bone tissue (transparent structure) and overlying cartilage layer (purple structure). (d) A thickness map of the cartilage layer (inferior view; 150 slices width) is presented as a pseudocolour-scaled image (*i.e.* blue to red; increasing cartilage thickness).

Figure 6 Three-dimensional articular thickness measurements were performed for the three groups of human samples. Differences were found between the average thicknesses of the cartilage layers between the groups. Furthermore, local differences in cartilage thickness were evident when the groups were compared. OA, osteoarthritis.

There was a significant difference in cartilage thickness between healthy and OA-early samples ($p = 0.032$) and between OA-early and OA-late samples ($p = 0.047$). Furthermore, thickness variance was significantly different between healthy and OA-late samples ($p = 0.047$). These local thickness changes could be visualized by producing thickness maps (Figure 6).

Discussion

The great advantage of the EPIC- μ CT technique is the 3D measurement ability. The aim of this study was to extend the application of the EPIC- μ CT technique for morphological bone–cartilage research in human and small animal TMJs. We are the first to apply EPIC- μ CT in this specific joint. The clinical relevance of this μ CT research lies in the possibility to apply the technique to model systems for diseases that also occur in humans (*e.g.* OA). This provides the advantage to accurately study the aetiology, the course of the disease, the effects of medication, etc. and provides the potential to further investigate the relationship between bone and cartilage changes in OA or other cartilage-related diseases in the jaw joint. All this gives a very thorough picture of how a disease progresses and how a treatment can work for human patients.

Previously, EPIC- μ CT was applied in the knee joint of a rat model.^{28,29} The cartilage thickness determined in these studies ranged from $403 \pm 11 \mu\text{m}$ in 4-week-old rats to $111 \pm 8 \mu\text{m}$ in 16-week-old rats. The accuracy for these thickness measurements was validated through precision evaluation (root-mean-square coefficient of

variation: 1.5%; root-mean-square coefficient of variation of standard deviation: $7.3 \mu\text{m}$) and by comparison with conventional needle probing ($r^2 = 0.95$, $p < 0.010$). Their power analysis indicated the possibility to detect a 1.3% thickness change with a sample size of 10 animals. In the present study, the average cartilage thickness found in the mouse TMJ showed a thickness range ($110 \pm 16 \mu\text{m}$) similar to the 16-week-old rats. The reliability of our findings was confirmed by a strong correlation between histology and EPIC- μ CT ($r^2 = 0.80$, $p < 0.0001$). From these results, we conclude that EPIC- μ CT is applicable for TMJ-OA research in a small animal model such as the mouse. There might be a crucial role for the SCB tissue in the process of OA. This fascinating concept needs to be investigated further; therefore, new challenges should be met, such as experimental *ex vivo* research, allowing studying the interaction between SCB and articular cartilage tissue. OA animal models can allow investigation of early disease progression and temporal changes in a relatively controlled environment; however, *ex vivo* research requires animal euthanasia at each desired interval. Future advances in non-invasive μ CT imaging can allow *in vivo* imaging in small animal joints with isotropic resolution between 6 and $10 \mu\text{m}$.

For the human TMJ samples, the required immersion time was determined by the implementation of a cumulative immersion sequence. A minimal immersion time of 1 h was determined, which is 30 min longer than the value used in the rat knee protocol.²⁸ Most probably, the larger dimensions (*i.e.* total volume) of the human TMJ might cause this difference. Another potentially relevant difference is the fibrocartilage layer, consisting

of a fibrous superficial and the subsuperficial fibrocartilaginous layer,⁴² in the TMJ contains less sGAGs.³⁸ The result is a different FCD of the cartilage layers in jaw and knee joint, respectively. As EPIC- μ CT attenuation is inversely proportional with this FCD, increased attenuation values in the human TMJ were expected. This likely caused the required adjustment of the immersion time for the human TMJ samples. Since we were only interested in possible adjustments of the protocol for human TMJ samples and the potential ability for (local) cartilage thickness measurements in both human and mouse samples, cartilage changes such as GAG content was not evaluated in this study. Interestingly in the human samples, a significant increase in cartilage thickness was found between healthy joints and joints with early signs of OA (239.7 ± 50.5 and $414.2 \pm 122.6 \mu\text{m}$, respectively). A subsequent significant decrease was found in human joints with late signs of OA (197.4 ± 159.7). Increase in cartilage thickness during the earlier development of OA has been reported before.⁴³ It is hypothesized that in osteoarthritic cartilage, the integrity of the collagen network is lost, leading to more water absorption due to the presence of the proteoglycans.

To study the aetiology and progression of TMJ-OA, conventional μ CT can be applied for 3D bone reconstruction of the complete TMJ, including detailed morphology and the bone's mineral degree and distribution.^{21,22} Subsequently, EPIC- μ CT provides the 3D imaging of cartilage morphology and thickness measurements. As described before, the technique can also be used for measurements of relative sGAGs concentration and distribution,^{27,29,32,44} an indication of the healthy state of the cartilage. However, caution should be exercised when using this technique for analysis of samples fixed in formalin. The mouse samples were stored in fixation fluid and the human samples were harvested from embalmed subjects. Kotwal *et al*³² reported that a fixation procedure (pre-preservation *vs* post-preservation) does not influence the thickness or volume of the cartilage layer, and, furthermore, no significant changes in attenuation in the intact joint surface of a mouse model were found. In contradiction, Benders *et al*³¹ reported "that formalin fixation decreases X-ray attenuation levels in ioxaglate-stained cartilage", in bovine osteochondral plugs. Whether the fixation can influence the cartilage attenuation and thus sGAGs quantification is still on debate. In addition, no data are available on the influence of an embalming procedure on the attenuation levels.

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5. In TMJ-OA, the mechanical overload has been suggested as a predominant initiator of the disease.^{6,7,14} μ CT-based biomechanical computational models (*i.e.* finite element models) have become a standard tool to examine the mechanical properties of bone and to predict its mechanical behaviour.⁴⁵ Generally, when these dynamic models are used to examine the mechanical behaviour of cartilage, a uniform articular cartilage thickness of 100 or 200 μm has been assumed.^{46,47} However, our results indicate a wider range, including different thickness variances (Figure 6), more consistent with earlier findings in the human TMJ.⁴⁸ Morphological measurements of both bone and cartilage tissues *via* conventional μ CT and EPIC- μ CT could provide more accurate future biomechanical models. Besides this advantage, the EPIC- μ CT technique has the ability to determine the relative sGAGs concentration throughout the cartilage layer, since the attenuation values are considered inversely proportional to the distribution and average amount of sGAGs.^{27,29} These sGAG concentrations are linked to the biomechanical properties of the TMJ's cartilage layer⁴⁹ and can be implemented into a model, further improving the input of material and/or tissue properties.⁵⁰ For example, it becomes possible to determine how changes in bone composition and structure affect the mechanical properties of the SCB and thus whether these changes affect the overlying cartilage in a healthy or diseased joint using finite element analysis.

To conclude, we established the *ex vivo* application of a semiquantitative, high-resolution, 3D imaging technique (*i.e.* EPIC- μ CT) to assess the cartilage morphology in mouse and human TMJs. For the human TMJ the original protocol provided by Xie *et al*²⁹ had to be adjusted and the immersion time was set to 1 h. Conventional μ CT and EPIC- μ CT could allow for bone-cartilage interaction research in the initiation and progression of TMJ-OA in mouse and human models. The results in this study are encouraging for *ex vivo* research; however, *in vivo* application of the EPIC- μ CT technique in the jaw joint is challenging, and systematic further studies are needed to reveal if this technique has any clinical potential.

Acknowledgments

The authors thank Hans Korfage, Peter Brugman, Marion van Duin and Ton Bronckers for their technical assistance. We thank Cindy Cleypool and Teun de Vries for providing help with sample collection. Furthermore, many thanks go to Denise Duijster for critically reading this manuscript.

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