Calcified cartilage revealed in whole joint by X-ray phase contrast imaging

Ludovic Broche, Bertrand Favier, Hélène Rougé-Labriet, Sabine Drevet, Bernard Lardy, Emmanuel Brun, Benjamin Lemasson

Objective: X-ray Phase Contrast Imaging (PCI) is an emerging modality that will be in the next few years available in a wider range of preclinical set-ups. In this study, we compare this imaging technique with conventional preclinical modalities in an osteoarthritis mouse model.

Method: Phase contrast technique was performed on 6 post-mortem, monoiodoacetate-induced osteoarthritis knees and 6 control knees. The mice knees were then imaged using magnetic resonance imaging and conventional micro computed tomography. Examples of imaging surrogate markers are reported: local distances within the articular space, cartilage surface roughness, calcified cartilage thickness, number, volume and locations of osteophytes.

Results: Thanks to PCI, we can show in 3D calcified cartilage without contrast agent by a non-invasive technique. The phase contrast images reveal more details than conventional imaging techniques, especially at smaller scales, with for instance a higher number of micro-calciifications detected (57, 314 and 329 for MRI, conventional micro-CT and phase contrast imaging respectively). Calcified cartilage thickness was measured with a significant difference (p < 0.01) between the control (23.4 ± 17.2 μm) and the osteoarthritis induced animal (46.9 ± 19.0 μm).

Conclusions: X-ray phase contrast imaging outperforms the conventional imaging modalities for assessing the different tissue types (soft and hard). This new imaging modality seems to bring new relevant surrogate markers for following-up small animal models even for low-grade osteoarthritis.

1. Introduction

Osteoarthritis (OA) represents the most widespread osteoarticular disease. It constitutes a growing public health problem, with our aging population and expanding obesity wave. Nowadays it represents the most common cause for disability with consecutive annual health care costs of up to 128 billion dollars in the US [1,2]. New therapeutic and symptomatic options for cartilage diseases have been tested in the past years in animal models and clinical, such as anagisic treatment [3], injection of monoclonal antibody [4-6] or low dose radiotherapy [7]. Imaging modalities are becoming a gold standard to quantify the effectiveness of such therapies. Thus, it is crucial to develop, robust and reproducible joint imaging techniques with a high spatial resolution and sensitive to soft and hard tissues. Current clinical and preclinical imaging modalities have limitations in the detection of early cartilage and bony changes. Indeed, conventional X-ray absorption based Computed Tomography (CT) allows clear visualization of bone tissues but provides reduced sensitivity to soft tissues [8]. Cartilage is indeed poorly visible in conventional micro-CT and it mainly shows advanced and irreversible cartilage degradation resulting in joint space narrowing. Changes in the composition of joint cartilage or soft tissues are usually rather evaluated using Magnetic Resonance Imaging (MRI) using sequences such as ultrashort-TE [9]. Yet, images obtained by MRI struggle to render properly bony changes and microcalciifications [10]. Indeed, MRI cannot so far reach the few microns spatial resolution required to characterize properly microcalciifications. Fluorescence imaging is also used to quantify the distribution of specific chemical elements such as reactive oxygen species but is limited to imaging at the macro scale and in two
dimensions [11].

Using a wave approach with X-rays, the refraction index of light element materials can be a thousand times greater than its counterpart the absorption factor for the wavelength in radiology [12]. This induces a much greater contrast for soft tissues with X-ray imaging methods based on the detection of the light refraction or with a wave description the phase [13]. These methods, called Phase Contrast Imaging (PCI) in comparison to the conventional method based on absorption. PCI has a strong correlation with MRI in measuring cartilage thickness and with conventional CT in detecting subchondral bone changes [14,15]. It is indeed possible to visualize simultaneously and in details the high absorbing tissues like bone, and the low-absorbing tissues like cartilage and soft tissue (synovial membranes, muscles ...) as demonstrated by Hornig [14]. Nevertheless, to our knowledge, only a few studies evaluated PCI for tissue (synovial membranes, muscles tissues like bone, and the low-absorbing tissues like cartilage and soft tissue) as demonstrated by Hornig [14].

The objectives of this study are to present the very first results obtained using PCI on a murine OA model and to evaluate the PCI capability to depict soft and hard tissues of the knee compared to the established preclinical imaging modalities. We also propose several new 3D morphometric surrogate markers with a special focus on articular cartilage, calcified cartilage and osteophytes.

2. Methods

2.1. Overall protocol

All imaging protocols were done post-mortem on twelve mice in which we analyzed both knees \((n_{\text{control}} = 6, n_{\text{OA}} = 6)\). The removed knees were formalin fixed, agarose imbedded, and synchrotron phase contrast imaging (PCI) was performed, followed by MRI and conventional x-ray microscopy. Then samples were then sliced for histological analysis.

2.2. Animal manipulation

In accordance with the Directive 2010/63/EU, the experiments were performed in an agreed animal facility (C3851610006) evaluated by an Ethical Committee for Animal Welfare and authorized (APAFIS #13792–201802261434542 v3). OA pain model was induced by single intra-articular injection of monoiodoacetate (MIA-OA, Sigma, Saint Quentin-Fallavier, France) in the knee. Six 6 weeks old C57Bl/6 female mice (Janvier Labs, France) were anaesthetized by xylazine and ketamine and killed. Knees were removed, formalin embedded; 4 mm embedded. The number of projections was 3600.

2.3. Synchronous X-ray imaging

Image acquisition was performed at the biomedical beamline of the ESRF (Grenoble, France). A propagation-based PCI technique was used to enhance contrast in between small density changes by capitalizing on coherent radiation, with a long sample to detector distance (11 m) to both x-ray absorption and refraction. This imaging technique uses highly coherent radiation, with a wavelength in radiology [12]. This induces a much greater contrast for soft tissues with X-ray imaging methods based on the detection of the light refraction or with a wave description the phase [13]. These methods, called Phase Contrast Imaging (PCI) in comparison to the conventional method based on absorption. PCI has a strong correlation with MRI in measuring cartilage thickness and with conventional CT in detecting subchondral bone changes [14,15]. It is indeed possible to visualize simultaneously and in details the high absorbing tissues like bone, and the low-absorbing tissues like cartilage and soft tissue (synovial membranes, muscles ...) as demonstrated by Hornig [14]. Nevertheless, to our knowledge, only a few studies evaluated PCI for tissue (synovial membranes, muscles tissues like bone, and the low-absorbing tissues like cartilage and soft tissue) as demonstrated by Hornig [14].

The objectives of this study are to present the very first results obtained using PCI on a murine OA model and to evaluate the PCI capability to depict soft and hard tissues of the knee compared to the established preclinical imaging modalities. We also propose several new 3D morphometric surrogate markers with a special focus on articular cartilage, calcified cartilage and osteophytes.

2.4. Magnetic resonance imaging

MRI was performed at 9.4T in a horizontal bore magnet (Bruker Biospec 94/20 Avance III-HD, Bruker BioSpec, Ettlingen, Germany) at the IRMaGe MRI facility (La Tronche, France) equipped with an helium cooled cryoprobe and a phased-array surface receive coils configuration. A 3D ultra-short echo time (UTE) pulse sequence was acquired (echo time \(= 0.00813\) ms; repetition time \(= 4.0\) ms; flip angle \(= 5^\circ\); acquisition matrix \(= 300 \times 300 \times 300\), average \(= 4\)). Such acquisition allows acquiring a volume of \(15 \times 15 \times 15\) mm \(^3\) at a voxel size of \(50 \times 50 \times 50\) \(\mu\)m \(^3\) in 17 min.

2.5. Conventional X-ray microtomography

Samples were imaged on a conventional microtomograph (EasyTom XL from RXSolution, Chavanod, France) at the SImaP- GPM2 lab (Grenoble, France). A conventional microfocus X-ray tube was used, with a maximum high voltage set to 60 kVp coupled with a 127 \(\mu\)m flat panel detector allowing for an acquisition time of 11 min for a \(11.1 \times 11.1 \times 8.8\) mm \(^3\) volume at a voxel size of 6.06 \(\mu\)m \(^3\). The source spot size was adjusted to be inferior to 5 \(\mu\)m. The number of projections was 3600.

2.6. Histological analysis

Bone and calcified cartilage structure were segmented using a region-growing algorithm [17]. A morphological opening was then used to remove structures smaller than 3 voxels in diameter [18]. All segmented elements (bone and calcified cartilage), not connected to the tibia, femur, fibula, menisci and patella were labelled as calcifications, for which we reported their number and volumes. The marching cube algorithm [19] was applied to every segmented structure to generate the polygonal surface meshes. The marching cube algorithm [19] was applied to every segmented structure to generate the polygonal surface meshes. A feature preserving mesh smoothing [20] algorithm was then applied to remove aliasing artifacts. Distance map was calculated to grade the severity of OA [16]. This semi-quantitative scoring system ranged from 0 to 6 (the higher the more severe) and was applied to all four quadrants (score out of 24): medial femur, medial tibia, lateral femur, lateral tibia.

2.7. PCI processing and analysis

Bone and calcified cartilage structure were segmented using a region-growing algorithm [17]. A morphological opening was then used to remove structures smaller than 3 voxels in diameter [18]. All segmented elements (bone and calcified cartilage), not connected to the tibia, femur, fibula, menisci and patella were labelled as calcifications, for which we reported their number and volumes. The marching cube algorithm [19] was applied to every segmented structure to generate the polygonal surface meshes. A feature preserving mesh smoothing [20] algorithm was then applied to remove aliasing artifacts. Distance map was calculated to grade the severity of OA [16]. This semi-quantitative scoring system ranged from 0 to 6 (the higher the more severe) and was applied to all four quadrants (score out of 24): medial femur, medial tibia, lateral femur, lateral tibia.
2.8. Statistics

Pairwise comparisons were performed by using independent Student-Newman–Keuls tests on each condyle interspace with the tibia, in between experimental conditions. When both condyles were included in one tested group a Kolmogorov-Smirnov test was applied to consider the distributions bimodality. Asymmetric aperture map distributions were pairwise tested in between conditions using a t-test. Finally, a Levene’s test was used to test pairwise comparison of the curvature map standard deviations. All statistical tests were carried out with a significance level of $p < 0.01$ using the SciPy statistical library with the Python3.7 language [24,25].

3. Results

3.1. Comparison of the phase contrast imaging technique to MRI, X-ray micro-tomography

Fig. 1 shows a representative slice, extracted from each 3D volumes, of a MIA-OA mice knee. MRI (Fig. 1A) with a ultra-short echo time sequence shows a readable contrast between bone and tissue structure as well as within the tissue structures (tendons, muscles, cartilages). But, the lack of image definition (pixel size of 50 μm [3]) gives a poor geometric representation of the overall bone, meniscus and tibial ligaments and underestimate the total number of calcifications ($N_{calcification_{MRI}} = 57$, $N_{calcification_{CT}} = 314$ vs $N_{calcification_{PhaseContrast} = 329}$). Due to poor contrast within the bone structure, calcified cartilage, cortical and trabecular bone appear homogeneous with the MRI technique. Conventional X-ray micro-tomography (Fig. 1B) with its high resolution shows a precise morphology of the bone structure but completely lacks contrast within soft and bony tissues. PCI (Fig. 1C) allays a high image precision good differentiation between all anatomical elements of the knee joint (muscle, tendon, calcified cartilage, articular cartilage, bone and calcifications). The insets shown in Fig. 1D and E are situated in the joint space and show the same zone with adapted gray scales for enhancing either the articular cartilage and synovial fluid (Fig. 1D) or the calcified cartilage (Fig. 1E). On these example, one can clearly see, in the same imaging modality, all the element of the knee joint: cartilage, calcified cartilage and chondrocytes. All the imaging dataset for the leg imaged with the different modalities are available at doi://10.17605/OSF.IO/W4P6M.

3.2. Validation of PCI results with histological images

To validate our PCI data with the gold standard histology, we present in Fig. 2, a comparison on a representative slice of a control (Fig. 2A,C,E,G,H) and a MIA-OA (Fig. 2B,D,F,I,J) mice knees acquired by PCI (Fig. 2A–D) and safranin-0 colored histological sections (Fig. 2E–J) with a focus on the medial femoral condyle area (C,D,J). To highlight the unique capabilities of PCI to render such contrast, the PCI contrast window was set to highlight the density changes within the calcified structures. The articular cartilage is therefore too dark to be visible and we indicate by a red dotted line its boundaries found thanks to a segmentation based on another contrast windowing (see method section). The transition between the subchondral bone (κ) and the calcified cartilage (*) can be visualized with PCI on both the control and the MIA slices. Dark rounded dots, visible within the calcified cartilage in PCI are ascribed to chondrocytes (γ). In the knee histological sections, the purple safranin-O stains the cartilage peptidoglycans. The calcified cartilage can be found on these images by a reddish color that seem to be correlated with the PCI images in both the control and MIA images. In the MIA-treated knee, osteoarthritis is assessed by disorganization and broadening of the cartilage, safranin-O negative areas at the surface (Fig. 2I), clefts at the cartilage surface with dead cell ghosts (Fig. 2J). These features can also be seen on the PCI slices, with a thicker calcified cartilage layer and with darker and larger chondrocytes.

3.3. 3D histomorphometry analysis of two joints

Fig. 3 and Fig. 4 show representative examples of the different surrogate markers PCI may provide on both the control and MIA knee joints. After the segmentation of our images in bone and calcified cartilages zones we can measure virtual 3D histomorphometry quantities that are non-accessible with gold standard 2D histology.

The Fig. 3 presents rapid and direct 3D measurements of features similar to those used in human X-ray imaging or in histological evaluation of OA. We calculated on our segmented bone structures the mean femur to tibia inter-space at the condyle positions in three dimensions (Fig. 3A). The regions of interest where the mean distance and their standard deviation were calculated are indicated by the gray square. It is interesting to note that by averaging the mean inter-distance of both condyles no statistical differences appears in between the Control and the MIA joints (56.7 ± 8.5 μm vs 57.4 ± 17.3 μm) (Plain color bars in Fig. 3D). But the MIA-OA knee displays a destabilization between his two
condyles with a thin medial femur/tibia interspace (39.4 ± 7.1 μm) and a large lateral femur/tibia interspace (70.3 ± 6.5 μm), while such asymmetry is not present on the control knee (54.2 ± 8.5 μm vs 58.2 ± 8.5 μm).

Thanks to a granulometry algorithm [26], we can calculate in three dimensions the local thickness of the different tissues. The Fig. 3B shows on a sagittal cross-section the distribution of the local thickness whilst the Fig. 3C represents those values projected in 3D on the segmented surfaces. The full distribution of calcified cartilage thickness in both femur and tibia for each condition is shown in the lower panel E and F. The thickness analysis on the calcified cartilage shows an overall increased in thickness with the MIA-OA knee 46.9 ± 22.4 μm compared to the control 23.4 ± 17.2 μm thickness. Bone wise, the femur and tibia calcified cartilage mean thickness are 47.6 ± 15.7 μm and 46.3 ± 22.4 μm respectively on the MIA-OA knee, and 28.7 ± 18.6 μm and 18.1 ± 13.9.
μm respectively in the control knee. No statistical differences were found in between the femur and tibia on each of the two conditions for this mouse. We can nevertheless note that the local thickness is the largest in the MIA joints within the condyles where the femur/tibia interspace is the smallest. The total volume of MIA calcified cartilage in the full joint is also largely superior with $V_{\text{MIA}} = 9.42 \times 10^{-2} \text{ mm}^3$ vs $V_{\text{Ctrl}} = 6.35 \times 10^{-2} \text{ mm}^3$.

Additionally, we computed three-dimensional based measurements on the PCI images that are not accessible with histology. We first computed the surface roughness (estimated as the local curvature of a surface) of different interfaces: the calcified cartilage/articular cartilage interface and the calcified cartilage/subchondral bone interface. The Fig. 4A-D shows such measurements where the roughness is projected onto the surfaces (represented in false colors) of both the calcified cartilage (A and C) and subchondral bone (B and D) in an example of control (A and B) and MIA (C and D) joints. The subfigure 4E-H, together with an exemplary image, shows the roughness distributions restricted to the condyles positions. Fig. 4E and F is the measurement performed on the femur, and G and H on the tibia. Note that only the femur subchondral bone displays a significant increase in curvature variability on the MIA-OA model ($\sigma_{\text{CurvOA}} = 0.143 \mu\text{m}^2$; $\sigma_{\text{CurvCtrl}} = 0.075 \mu\text{m}^2$).

Fig. 4I-L shows the 3D distribution of all the segmented osteophytes in both control (I) and MIA (J) where the osteophytes were colored. Osteophytes were defined as high density elements not connected to the three main bones or the menisci. The graph in Fig. 4K and L, shows a 2D projections in sagittal and transverse coordinates where the volume of the osteophytes is schematized by a circle with the same volume. As one can see, the control knee presents some osteophytes probably due to the injection of PBS but the MIA-OA knee displays a significantly higher amount ($n_{\text{OA}} = 31$ vs $n_{\text{Ctrl}} = 13$) with a larger total volume ($V_{\text{OA}} = 4.29 \times 10^{-2} \mu\text{l}$ vs $V_{\text{Ctrl}} = 6.01 \times 10^{-2} \mu\text{l}$).

3.4. Quantification of the whole image database

All the computed metric results are recapitulated in Table 1. Fig. 5 displays the comparison of the morphological parameters computed in the same fashion of the above examples on the PCI images but for the all the MIA injected legs ($n = 6$) and their controlateral legs ($n = 6$). Fig. 5A presents the mean inter-space distance between femur and tibia taking into account both condyles (bars in plain color). The distance is significantly higher ($p$-value $< 0.01$) for the control than for the MIA knees. The group displays the same trend as in the above example when measuring specifically the interspace in between condyles. The same strong destabilization appears in between the MIA medial and lateral condyle ($55.6 \pm 9.1 \mu\text{m} \text{ vs } 56.7 \pm 4.1 \mu\text{m}$) while in the control group such effect is statistically non relevant ($55.6 \pm 9.1 \mu\text{m} \text{ vs } 56.7 \pm 4.1 \mu\text{m}$).

The same trend can be observed when measuring the calcified cartilage thickness (Fig. 5B and C). The MIA model animals display a significant higher thickness compared to the control for both the femurs (24.2
The surfaces roughness at the calcified and articular cartilage interface shows a smooth surface along the control and the MIA group for both the femur and tibia (Fig. 5D and F). On the other hand, as shown in Fig. 5E and G, the roughness on the tidemark between the calcified cartilage and subchondral bone is significantly different in between the two measured groups with a larger variability in curvature surface for the MIA model for both the femur and tibia.

The calcification distribution is significantly different between the model and the control (nbMIA = 54.8 ± 21.5 vs nbCtr = 12.1 ± 4.21) (Fig. 5H). The volumes distribution seems bimodal in the MIA model with a total of V_{MIA} = 2.77 × 10^3 [3]μL vs V_{MIA} = 3.70 × 10^2 [4]μL. As expected, the smallest calcifications are mainly distributed in the tightest spaces in between the two condyles.

The total volume of calcified cartilage was also higher in the MIA group compared to the control with a mean volume per animal of VMIA = 11.0 ± 2.45 × 10^-2 mm³ vs V_{Cr} = 5.84 ± 0.86 × 10^-2 mm³ [3].

4. Discussion

The main objective of this work was to study whether Phase Contrast Imaging is a suitable imaging modality for studying at high resolution a murine osteoarthritis model. We demonstrated that compared to conventional MRI and conventional micro CT images, PCI technique has a higher image sensibility and resolution. It allows in a single scan a full representation of the soft and calcified tissue structures at the same resolution than conventional micro-CT that is limited to bone visualization only. We showed for the first time the possibility to distinguish in 3D, using a non-invasive technique i.e. in complete unaltered mice knees, the articular and calcified cartilages as well as the subchondral bone and some clustered chondrocytes.

In this study, for sake of comparison with literature, we used the most characterized chemical-induced OA mouse model based on the injection into the rodent knee joint of mono-iodoacetate (MIA), an inhibitor of glyceraldehyde-3-phosphate dehydrogenase, a glycolysis enzyme [27]. Although not fully depicting the disease progression in human, this MIA-OA model induces chondrocytes cell death within a week, a rapidly evolving OA and formation of osteophytes in rodent [28]. The chemical
agents (Mono-Iodo-Acetate (MIA), papain, collagenase) induce very serious lesions with complete and rapid destruction of the cartilage making it impossible to study the early phases of OA disease. Despite that, to depict the joints tissue elements with enough contrast at the micro scale in such a small model remain highly challenging with conventional pre-clinical imaging modalities. In this study the histological lesions are severe and confirm that an OA has been induced. Some samples are close to the maximum of the OARSI scoring system with large depletion of proteoglycan staining, while other have a lack of articular cartilage. Mice calcified cartilage is proportionally larger in mice than in human [16] and mice MIA-OA model is mildly affected by mechanical stress due to the animal weight [27]. We nevertheless found thickening of the calcified cartilage and a rougher surface of the tidemark in the OA-model. As expected on an MIA-OA model [29], we also found a drastic increase in micro-calciﬁcation number and volume. Such metrics could potentially be a relevant biomarker to spot early stage of MIA-OA model. On the other hand, surface roughness of the subchondral bone and calcified cartilage tidemarks did not exhibit any pertinent outcomes on that model. We also observed no sign of subchondral bone alteration such as osteophytes, vascular inﬁltration or osteoclasts. In a hypothetical clinical usage of the PCI technique for osteoarthritic joint detection, calcified cartilage thickening could potentially join the already existing diagnostic radiographic hallmarks.

The secondary objective of this study was to present some surrogate markers this 3D imaging modality may offer. Besides the measurements of features classically admitted as hallmarks of OA (cartilage thickness, distance between bones), we proposed new morphological biomarkers to grade cartilage alterations, calculated here in 3D. These markers were

| Morphological Metrics | Control (n = 6) | MIA (n = 6) | p < 0.01 |
|-----------------------|----------------|-------------|----------|
| Interspace Femur/Tibia | 56.3 ± 6.6     | 37.4 ± 13.9 | * * *    |
| Media/Lateral Condyle | 55.6 ± 9.1     | 25.6 ± 6.8  |          |
| Local Thickness of the Tidemark | 24.2 ± 7.3 | 47.7 ± 15.4 | * *      |
| Curvature of the Subchondral Bone | 25.1 ± 8.1 | 44.4 ± 17.2 |          |
| Calcified Cartilage | 5.84 ± 0.86   | 11.0 ± 2.45 | *        |
| Calcified Cartilage Volume | 4.32 ± 1.09 × | 4.57 ± 1.72 × | **      |
| Calcified Cartilage Volume | 4.75 ± 1.14 × | 4.81 ± 1.82 × | **      |
| Calcified Cartilage Volume | 6.78 ± 2.25 × | 12.7 ± 3.54 × |          |
| Calcified Cartilage Volume | 7.54 ± 2.47 × | 11.6 ± 2.85 × |          |
| Calcified Cartilage Volume | 10² ×          | 10²        |          |
| Calcified Cartilage Volume | 12.1 ± 4.21 | 54.8 ± 21.5 | *        |
| Calcified Cartilage Volume | 2.77 ± 0.462 × | 3.70 ± 0.684 × | *      |
| Calcified Cartilage Volume | 10³ ×          | 10²        |          |

Table 1
All computed metrics on the knees database (control n = 6 and MIA n = 6). With D: Interspace the tibia and the femur; T: local thickness of the calcified cartilage; V: volume; C: Curvature of the specified surface; N: Number of calcification.
calculated on the calcified cartilage due to the lack of articular cartilage on some animals of the MIA group, but in principles all these morphological parameters could have been measured on other tissues. In summary, we have shown that synchrotron radiation phase contrast imaging is a promising technique to rapidly detect and quantify microstructures for the investigation of small animal models of osteoarthritis. For example, the mean distance between the tibial plate and the femur is not different between the control and the MIA group but a difference can be observed if one measure separately the condyles. As expected, the calcified cartilage is thicker than in the OA group even in a OA low grade knee. The number and volume of calcifications seem to be a good indicator of the disease. The roughness of the interface between the calcified cartilage and the subchondral bone seem to be discriminant as well contrary to the interface between articular and calcified cartilage.

The main limitation of this study is the low number of investigated samples (n = 6). Future studies will be carried out in larger groups of animals and other mouse model (surgically induced for example) to further evaluate the usefulness of PCI discriminant markers in the OA diseases. This limitation is mainly due to the limited access to synchrotron. But as previously shown the PCI technique is perfectly compatible with the preclinical in-vivo applications [30, 31]. The next steps will consist in performing such experiment in-vivo on laboratory systems at a reduced radiation dose. Indeed synchrotron limited access, does not allow to follow-up the pathologies or at the cost of using many animals; while thanks to recent development performing such experiment on conventional X-ray source seems feasible in relatively short future. Indeed, the technique does not need any sample preparation or chemical injection and the deposited radiation dose could drastically be reduced with iterative reconstruction algorithm [32]. Latest development in PCI techniques using grating interferometry [33–35] has proven the feasibility of performing phase contrast with clinical sources. Unfortunately, in these studies the application of PCI remains in 2D due to the additional optics used. A simple approach using a random modulator [36–38] has recently shown that PCI can be transferred on conventional source with short acquisition time and reduced radiation dose to the sample [39].

To conclude, PCI provides in a single examination, a better and a more detailed depiction of the different tissues of small animal models. The sensitivity of PCI makes possible a diagnosis and staging tool of the joint deterioration to support the development of new pre-osteoarthritic treatments and could in the future be applied to clinical studies.

Author contributions

Study conception and design: LB, BF, HRL, SD, BLa, BLe, EB; Data acquisition: LB, BF, HRL, EB, BLe; Data analysis and interpretation: LB, BLe, EB; Drafting the article: LB, BF, HRL, SD, BLa, BLe, EB; Article final approval: LB, BF, HRL, SD, BLa,BLe, EB.

Declaration of competing interest

The authors declare no conflict of interests.

Acknowledgements

The authors acknowledge the ESRF for granting beamtime and their support during the experiment. Part of this study was funded by the Idex Grenoble grant IRS Valium. The authors acknowledge C. Ferrero for scientific discussion, L. Salvo P. Luijssier for beamtime and help on the conventional microCT device.

References

[1] N. Arden, M.C. Nevitt, Osteoarthritis epidemiology. Best pract, & Res. Clin. Rheumatol 20 (2006) 3–25, https://doi.org/10.1016/j.berh.2005.09.007 (Osteoarthritis).
[2] G.A. Hawker, Osteoarthritis is a serious disease, Clin. Exp. Rheumatol. 37 (2019) S3–S6.
[3] R.M. Stevens, et al., Randomized, double-blind, placebo-controlled trial of intraarticular trans-capacsin for pain associated with osteoarthritis of the knee, Arthritis Rheum. 71 (2019) 1524–1533.
[4] R. Fleischmann, et al., Upadacitinib versus placebo or adalimumab in patients with rheumatoid arthritis and an inadequate response to methotrexate: results of a phase iii, double-blind, randomized controlled trial, Arthritis Rheum. 71 (2019) 1798–1809.
[5] M. Kloppenburg, et al., Phase iia, placebo-controlled, randomised study of lutikizumab, an anti-interleukin-1 and anti-interleukin-1j dual variable domain immunoglobulin, in patients with erosive hand osteoarthritis, Ann. Rheum. Dis. 78 (2019) 413–420.
[6] Y. Jin, et al., Cgrp blockade by galanezumab was not associated with reductions in signs and symptoms of knee osteoarthritis in a randomized clinical trial, Osteoarthr. cartilage 25 (2018) 1609–1618.
[7] M. Minten, E. Mahler, A. den Broeder, J. Leer, C. van den Ende, Ab0864 the efficacy and safety of low-dose radiotherapy on pain and functioning in patients with osteoarthritis: a systematic review, 2015.
[8] S. Botter, et al., Quantification of subchondral bone changes in a murine osteoarthritis model using micro-ct, Biorehabilitation 43 (2006) 379–388.
[9] J. Du, G.M. Bydder, Qualitative and quantitative ultrashort-te mri of cortical bone, NMR Biomed. 26 (2013) 489–506, https://doi.org/10.1002/nbm.2906.
[10] M. Kloppenburg, et al., Cartogenin treatment prevented joint degeneration in a rodent model of osteoarthritis: a pilot study, J. Orthop. Res. 34 (2016) 1780–1789.
[11] L. Xie, et al., Quantitative imaging of cartilage and bone morphology, reactive oxygen species, and vascularization in a rodent model of 11. Xie, L. et al. Quantitative imaging of cartilage and bone morphology, reactive oxygen species, and vascularization in a rodent model of osteoarthritis, Arthritis Rheum. 64 (2012) 1899–1908.
[12] A. Bravin, P. Coan, P. Suzetti, X-ray phase-contrast imaging: from pre-clinical applications towards clinics. Phys. Medicine & Biol 58 (2013) R1–R35, https://doi.org/10.1080/00319155.2012.11.1/R1.
[13] T. Davis, D. Guo, T. Gureyev, A. Stevenson, S. Wilkins, Phase-contrast imaging of weakly absorbing materials using hard x-rays, Nature 373 (1995) 595–598.
[14] A. Horng, et al., Cartilage and soft tissue imaging using X-rays: propagation-based phase-contrast computed tomography of the human knee in comparison with clinical imaging techniques and histology. Investig. radiology 49 (2014) 627–634, https://doi.org/10.1097/RLI.0000000000000563.
[15] D. Paganin, S.C. Mayo, T.E. Gureyev, P.R. Miller, S.W. Wilkins, Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object,
[16] S.S. Glasson, M.G. Chambers, W.B. Van Den Berg, C.B. Little, The oarsi histopathology initiative 2013; recommendations for histological assessments of osteoarthritis in the mouse. Osteoarth. Cartil 18 (2010) S17–S23, https://doi.org/10.1016/j.joca.2010.05.025.

[17] B. Adams, L. Bischof, Seeded region growing, IEEE Trans. Pattern Anal. Mach. Intell. 16 (1994) 641–647.

[18] A. Anso, Y. Kobayashi, C. Muraki, M. Muneyasu, Optimization of gray scale morphological opening for noise removal in texture images, in: The 2004 47th Midwest Symposium on Circuits and Systems, 2004. MWSCAS ’04., vol. 1, IEEE, 2004. I–313.

[19] D. Rajon, W.E. Bolch, Marching cube algorithm: review and trilinear interpolation adaptation for image-based dosimetric models, Comput. Med. Imag. Graph. 27 (2003) 411–435.

[20] T.R. Jones, F. Durand, M. Desbrun, Non-iterative, feature-preserving mesh smoothing, ACM SIGGRAPH 2003 Papers (2003) 943–949.

[21] D. Man, K. Uda, H. Ueyama, Y. Ito, K. Nakano, Implementations of a parallel algorithm for computing euclidean distance map in multicore processors and gpus, Intl. journal networking computing 1 (2011) 260–276.

[22] E. Brun, J. Vicente, F. Topin, R. Occelli, Imorphe: a 3d morphological tool to fully analyse all kind of cellular materials, Cell. Met. for Struct (2008).

[23] B. Will Schroeder, L. Ken Martin, Visualization toolkit: an object-oriented approach to 3D graphics, 1996. Kitware, fourth ed.

[24] B. Falissard, Comprendre et utiliser les statistiques dans les sciences de la vie (DEPRECIATED), 2005.

[25] P. Virtanen, et al., Scipy 1.0: fundamental algorithms for scientific computing in python, Nat. Methods 17 (2020) 261–272.

[26] E. Brun, C. Ferrero, J. Vicente, Fast granulometry operator for the 3d identification of cell structures, Fundam. Inf. 155 (2017) 363–372.

[27] E.L. Kuyinu, G. Narayanan, L.S. Nair, C.T. Laurencin, Animal models of osteoarthritis: classification, update, and measurement of outcomes, J. orthopaedic surgery research 11 (2016) 19.

[28] P. Mapp, et al., Differences in structural and pain phenotypes in the sodium monoiodoacetate and meniscal transection models of osteoarthritis, Osteoarthr. cartilage 21 (2013) 1326–1345.

[29] G. Li, et al., Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes, Arthritis Res. Ther. 15 (2013) 223.

[30] M. Bech, et al., In-vivo dark-field and phase-contrast x-ray imaging. Sci, reports 3 (2013) 3209.

[31] L. Fardin, et al., Enhancing lung tumor visibility using in-vivo analyzer-based x-ray phase contrast imaging in mouse: a feasibility study, Am. J. Respir. Crit. Care Med. 195 (2017).

[32] Y. Zhao, et al., High-resolution, low-dose phase contrast X-ray tomography for 3D diagnosis of human breast cancers, Proc. Natl. Acad. Sci. United States Am. 109 (2012) 18290–18294, https://doi.org/10.1073/pnas.1204460109.

[33] F. Pfeiffer, et al., Grating-based x-ray phase contrast for biomedical imaging applications, Zeitschrift für medizinische Physik 23 (2013) 176–185.

[34] H. Yoshikawa, et al., Imaging evaluation of the cartilage in rheumatoid arthritis patients with an x-ray phase imaging apparatus based on talbot-lau interferometry, Sci. Rep. 10 (2020) 1–9.

[35] Z. Wang, et al., Non-invasive classification of microcalcifications with phase-contrast x-ray mammography, Nat. communica- tions 5 (2014) 1–9.

[36] D.M. Paganin, H. Labriet, E. Brun, S. Berujon, Single-image geometric-flow x-ray speckle tracking. Phys. Rev. A 98, 053815, 2018, https://doi.org/10.1103/PhysRevA.98.053815.

[37] H. Labriet, et al., 3D histopathology speckle phase contrast imaging: from synchrotron to conventional sources, in: Medical Imaging 2019: Physics of Medical Imaging, Vol. 10948, 109481S (International Society for Optics and Photonics, 2019).

[38] M.-C. Zdora, et al., X-ray phase-contrast imaging and metrology through unified modulated pattern analysis. Phys, review letters 118 (2017) 203903.

[39] H. Roug/Labo-Labriet, et al., Comparison of x-ray speckle-based imaging deflection retrieval algorithms for the optimization of radiation dose, Phys. Med. Biol. 66 (2020), 065005.