Contrast enhanced micro-computed tomography imaging of joint degradation in osteoarthritis rat models

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Aims

Osteoarthritis (OA) is the most common joint disease that affects all tissues within the joint. A histological gold standard, OARSI grading[1], evaluates degenerative features more at the superficial part of articular cartilage, and we have previously reported that bone thickening is associated with histopathological OA severity[2]. Bone provides natural contrast for micro-computed tomography (micro-CT), but imaging of articular cartilage has remained a challenge. To overcome this, we have previously validated a phosphotungstic acid (PTA) staining based contrast-enhanced micro-CT method for imaging of articular cartilage. PTA binds to collagen, which is the most prevalent protein in cartilage. This method not only can provide information on collagen content[3] but can also be used to reveal histological features in 3D[4]. In this study, we aimed to establish a standardized protocol for rat joint ex vivo imaging, and to quantify articular surface features due to cartilage degeneration using in-house-developed surface analysis tool.

Method

In the first part, we collected right knees from eighteen (*N*=18) healthy 11-month-old rats (permit number 31/2017). Femurs were divided into three groups. First group was kept as a native control. Second group was treated for 24h with chondroitinase ABC (Sigma-Aldrich, St. Louis, MO, C3667; 0.1 U/ml, at 37°C, 5% CO2) in phosphate-buffered saline with 0.01% bovine serum albumin to induce cartilage degeneration. The second and third group were subjected for 24h to lubricant molecule that binds to the cartilage surface and possibly can delay PTA diffusion. This lubricant is a graft copolymer based on poly(2-methyl-2-oxazoline)s (PMOXA) that was developed to restore lubricious properties of degenerating cartilage surface and to protect it from further enzymatic degradation. In the second part, four rats were used to test the protocol's capability for detecting surface degradation. Each rat was injected with 250U collagenase into the left knee to initiate osteoarthritis. Both injected and contralateral legs were collected at 4, 8, 12 and 16 days after injection.

All femurs were first fixed in 10% neutral-buffered formalin for 7 days and transferred to 70% ethanol 8 hours prior to staining with solution containing 1% w/v PTA and 70% ethanol. In the first part, micro-CT imaging was conducted for the samples in two-hour intervals from 8 to 16 and 14 to 22 hours for controls and lubricant treated samples, respectively. Micro-CT scanner's

(Skyscan 1272) X-ray tube was set to 50kV and 0.5mm aluminum filter was applied. Projection images were collected every 0.3˚ (over 360˚) and two frames were averaged from each projection. Images were reconstructed to 6.8 µm pixel size and ring artifact reduction of 8 and 30% beam hardening corrections were applied. PTA intake was analyzed as previously[3] with exception that thin rat cartilage was normalized between 0.0 and 0.6 instead of 0 and 0.8.
In the second part, to accommodate the need for higher resolution in the surface analyses, projection images were collected every 0.15˚ over 360˚ with frame averaging of three. Images were reconstructed to 2.8 µm pixel size and ring artifact reduction of 8 and 50% beam hardening corrections were applied were used. Similar PTA profiles were analyzed as in the first part by normalizing cartilage thickness and plotting vertical grey scale profiles. To quantify cartilage surface degradation, we used a custom-made Matlab algorithm where a reference surface is fitted on top of the cartilage surface and the volume between these surfaces is analyzed. Surface area of 1.4 x 2.5 mm was chosen from each condyle from the weight bearing area. This is followed by generating simple and complex surfaces. Complex surface follows precisely the actual surface morphology, whereas the simple surface is rather a projection, thus neglecting the complexity.The total volumes between the reference surface and these two surfaces were calculated and their ratio defines the complexity of the actual surface: a ratio of 1 defines a surface with no complex structures. An average distance of the shortest route is calculated by mapping the shortest path from the reference surface to the actual surface following the complex surface.
In the first part we collected right knees from eighteen (N=18) healthy 11 month old rats (permit number 31/2017). Femurs were divided to three groups. First group was kept as native control. Second group was treated for 44h with chondroitinase ABC (Sigma-Aldrich, St. Louis, MO, C3667; 0.1 U/ml, 44 h at 37°C, 5% CO2) in an aqueous 0.01% bovine serum albumin solution to cause cartilage degeneration. The second and third group were subjected for 24h to lubricant molecule that binds to the cartilage surface and possibly delays PTA diffusion.
In the second part four rats were used to test protocol capability for detection of surface degradation. One rat was kept as control and three rats got collagenase injection to left knee to cause osteoarthritis. From collagenase group both legs were collected 8, 12 and 16 days after injection.
All femurs were first fixed in 10% neutral-buffered formalin for 7 days and transferred to 70% ethanol 8 hours in prior to staining with solution containing 1% w/v PTA and 70% ethanol. Samples for first part were microCT imaged in two hour intervals from 8 to 16 and 14 to 22 hours for controls and lubricant treated samples, respectively. Scanner (1272, Bruker MicroCT) X-ray tube was set to 50kV and 0.5mm aluminiun filter was applied. Projection images were collected every 0.3 deg over 360 deg and frame averaging of 2 was applied. Images were reconstructed to 6.8um pixel size and appropriate corrections were applied.
PTA intake was analyzed as previously[3] with exception that thin cartilage in rats was normalized between 0.0 and 0.6 instead of 0 and 0.8.
To accomodate the need for higher resolution in surface analyses projection images were collected every 0.15deg over 360deg with frame averaging of three. Images were reconstructed to 2.8 um pixel size and appropriate corrections were used. Similar PTA profiles were analyzed as for first series by normalizing cartilage thickness and plotting vertical grey scale profiles. Enzymatic cartilage degradation occurs first in the cartilage surface. To quantify these changes, we used a custom made matlab algorithm where a reference surface is fitted on top of the cartilage surface and the volume between these surfaces is analyzed. A 1.4x2.5mm surface area was chosen from each condyle from the weight bearing area. A simple surface detection does not take into account the possible complex structures in the surface. In addition to the actual cartilage surface, a simple surface is mapped where the complex structures are not included. The total volumes between the reference surface and these two surfaces are calculated and their ratio defines the complexity of the actual surface where a ratio of 1 defines a surface with no complex structures. An average distance of the shortest route from the reference surface to the actual surface is also calculated and it defines the average distance

between the two surfaces. This calculation is done voxelwise by mapping the shortest path in 3D between adjacent voxels in the reference surface and the actual surface.

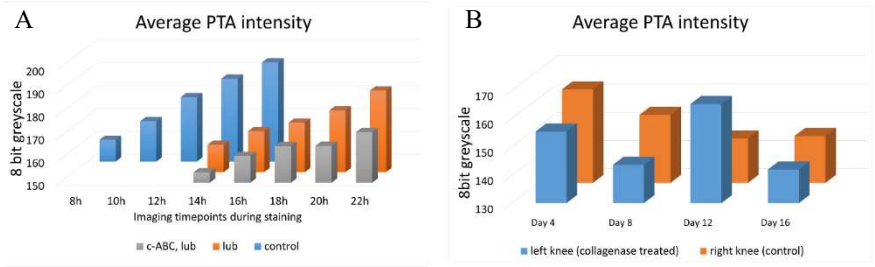

Figure 1. PTA diffusion into articular cartilage is reflected by increased absorption, which is shown here as increased grey scale values. A) Average PTA intensity is shown at different time points for all three groups - control (blue), lubricant (orange) and *in vitro* simulated OA treated with lubricant (grey). B) PTA content in articular cartilage of femoral condyles in left (injected) and right (control) knee. Decreased collagen content is seen already 8 days after injection (blue). However, 12 days after injection there was increased PTA content in the sample that also showed highest surface degradation.

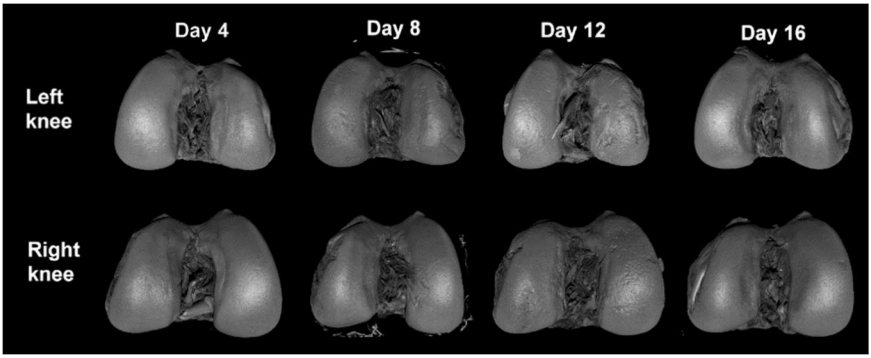

Figure 2. Surface visualizations of femoral condyles at different time points after collagenase injection (left knee) and control (right knee). This figure shows surface fibrillation at day 8 and highest surface degradation at day 12 in this early osteoarthritis model.

Results

PTA diffusion (Figure 1A) was significantly faster in control samples compared to lubricant treated samples. In control samples, complete staining was achieved after 14 hours, while similar level of PTA intake was achieved by 22 hours of staining in lubricant-treated samples. In samples that were first treated with chondroitinase ABC and then with lubricant, PTA staining was stabilized after 18 hours. However, in this group we observed somewhat surprising decrease in PTA intake.

In the second group with collagenase injection, there is a reduction in PTA uptake in injected knees compared to contralateral knees (Figure 1B). With exception in one sample with extensive surface erosion, where PTA uptake was increased.

Collagenase injections caused cartilage erosion which was already visible by 8 days after injection (Figure 2). Greatest benefit from our surface analysis tool is that it can be applied to complex surfaces. Shortest route to surface (Figure 3A) and complex volume (Figure 3B) of cartilage lesions increase already at day 8 and both these parameters show disease progression with time. This disease progression is especially clear when looking at surface complexity ratio (Figure 3C).

Figure 3. An average A) shortest route to surface and B) complex volume in cartilage lesions and C) surface complexity ratio at different time points after collagenase injection. Both shortest route to surface and complex volume show provide quantitative information of surface degradation and correspond with visual images. The surface complexity ratio indicates gradual disease progression in injected knee when comparing to control knees.

Conclusion

Our aim is to provide sensitive imaging biomarkers to evaluate osteoarthritis severity and effectiveness of osteoarthritis treatments in pre-clinical rat models. We propose that rat knees should stained for 20h with PTA to minimize any possible effects in histological analyses. With sufficient resolution (< 3 µm) key surface features can be identified and quantified with presented analysis methods.

References:

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