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Contrast-enhanced micro-computed tomography of articular cartilage morphology with ioversol and iomeprol

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CECT imaging using ioversol and iomeprol

Abstract

Non-ionic, low-osmolar contrast agents (CAs) used for computed tomography, such as Optiray (ioversol) and Iomeron (iomeprol), are associated with reduced risk of adverse reactions and toxicity in comparison to ionic CAs, such as Hexabrix. Hexabrix has previously been used for imaging articular cartilage but has been commercially discontinued. This study aims to evaluate the efficacy of Optiray and Iomeron as alternatives for visualisation of articular cartilage in small animal joints using contrast-enhanced micro-computed tomography (CECT). For this purpose, mouse femora were immersed in different concentrations (20% - 50%) of Optiray 350 or Iomeron 350 for periods of time starting at five minutes. The femoral condyles were scanned *ex vivo* using CECT, and regions of articular cartilage manually contoured to calculate mean attenuation at each time-point and concentration. For both contrast agents, a 30% contrast agent concentration produced a mean cartilage attenuation optimally distinct from both bone and background signal, whilst 5-minute immersion times were sufficient for equilibration of CA absorption.

Additionally, plugs of bovine articular cartilage were digested by Chondroitinase ABC to produce a spectrum of glycosaminoglycan (GAG) content. These samples were immersed in CA, and assessed for any correlation between mean attenuation and GAG content. No significant correlation was found between attenuation and cartilage GAG content for either CAs. In conclusion, Optiray and Iomeron enable high-resolution morphological assessment of articular cartilage in small animals using CECT, however, they are not indicative of GAG content.

Keywords:

Optiray, Iomeron, cartilage imaging, EPIC-μCT, CECT

Introduction

Osteoarthritis (OA), a chronic degenerative joint disease, is the most common joint disorder. The increasing prevalence of OA and a desire to start treatment as early as possible highlights the need for non-invasive accurate detection of joint damage at early stages of disease. This would minimise disability and pain and improve quality of life (Silvast et al., 2009).

To detect structural changes in articular cartilage, there is need for morphological assessment of the extracellular matrix (ECM). Needle probing, histology and stereophotographic techniques are readily available for this purpose, however, they provide limited information, and can be destructive and time-consuming. (Xie et al., 2009) Therefore, non-destructive medical imaging alternatives including micro-Magnetic Resonance Imaging (microMRI) and micro-computed tomography (microCT), are often used to visualise cartilage. (Watrin et al., 2001, Roberts et al., 2003, Watrin-Pinzano et al., 2005) Since MRI has a limited spatial resolution for mouse cartilage, focus has been shifted to microCT, which accomplish higher spatial resolution in a shorter acquisition time (Xie et al., 2009, Bansal et al., 2010, Lin et al., 2016, Willett et al., 2016, Lakin et al., 2016, Stok et al., 2016, Das Neves Borges et al., 2014).

Micro-CT is an x-ray based imaging technique, providing quantitative 3D analysis of tissues and is widely used for microstructural analysis of mineralised tissues, such as bone. Although microCT is a standard modality for preclinical bone assessment, (Laib et al., 2000, Bouxsein et al., 2010) its usage for articular cartilage is limited due to the low effective atomic number of the tissue, which results in a low attenuation of x-rays, and difficulty in distinguishing cartilage from surrounding soft tissues (Lusic and Grinstaff, 2013). Using a contrast agent (CA) that diffuses into the cartilage, it can compensate for poor radiopacity of cartilage and enables its visualisation by microCT. Visualisation and detection of OA-induced structural changes in articular cartilage of small animals is beneficial for investigating treatment and prevention methods, which can potentially be translated to the human disease.

Many CAs, such as iodine- or metal-based agents, possess elements of high atomic number – capable of greater X-ray absorption (Lusic and Grinstaff, 2013). Iodine-based CAs are widely used in different forms; they are High-Osmolar Contrast Media (HOCM) or Low-Osmolar Contrast Media (LOCM), ionic or non-ionic, and monomeric or dimeric. HOCMs have higher risk of inducing toxic effects and CA-induced nephropathy than LOCMs (Aspelin, 2006).

Hexabrix (Ioxaglate) is an example of an ionic iodine-based LOCMs, which was used for clinical (angiography) imaging. Hexabrix was first introduced for preclinical CECT imaging in 2006, (Palmer et al., 2006) and has not only enabled visualisation of the cartilage tissue by microCT, but also quantification of morphological features such as cartilage thickness and volume (Xie et al., 2012, Kerckhofs et al., 2013), (Kotwal et al., 2012). It was also used for assessment of articular cartilage sulphated glycosaminoglycan (sGAG) content, one of the main components of cartilage ECM (Xie et al., 2009).

Proteoglycans (PGs) are ECM components that compromise approximately 5-10 % of the wet weight of articular cartilage, (Palmer et al., 2006) and their loss is an early indicator of OA. (Buckwalter and Mankin, 1998) Since most ionic iodinated-based CAs (such as Hexabrix) are negatively charged, the CAs and negatively charged side groups of the PGs in cartilage repel each other, resulting in poorer absorption of the CA into cartilages with high GAG content. This shows as a negative correlation between CECT attenuation and GAG content, suggesting the use of ionic CAs as a predictor of GAG content in articular cartilage (Bansal et al., 2010, Xie et al., 2010).

Although Hexabrix was used by researchers for preclinical cartilage imaging, this was not the primary market. It was recently discontinued by the manufacturer and replaced by other existing agents for clinical angiography, such as Optiray (ioversol).(Food and Drug Administration) Optiray (Ioversol; $C_{18}H_{24}I_3N_3O_9$; Figure 1a)(National Center for Biotechnology Information) and Iomeron (Iomeprol; $C_{17}H_{22}I_3N_3O_8$; Figure 1b) (National Center for Biotechnology Information) are two commercially available non-ionic iodinated LOCMs. Non-ionic CAs are estimated to yield 5 times fewer adverse reactions than ionic agents, such as Hexabrix, and this makes them a better candidate for CECT (Aspelin, 2006), (McClennan, 1990, Christiansen, 2005).

In this study, we show the efficacy of Optiray and Iomeron for articular cartilage visualisation by CECT. The primary aim of this study is to assess the viability of these two contrast agents for *ex vivo* evaluation of cartilage morphology in mouse femoral articular cartilage using CECT. The second aim of this study is to determine whether a correlation exists between GAG content and CECT attenuation using Optiray or Iomeron.

Materials & Methods

Specimen preparation

For assessment of articular cartilage morphology with Optiray and Iomeron, femora were dissected from excess 10-week old BALB/c mice carcasses from the Biomedical Sciences Animal Facility at the University of Melbourne (n=12 per group). After removing the surrounding soft tissues, the samples were stored in phosphate buffered saline (PBS) containing 1% protease inhibitor, at 4°C.

For assessment of CA attenuation, Ø5 mm plugs were cored from bovine femoral cartilage acquired from an abattoir (n = 1, retired breeder). To create a spectrum of GAG content within the cartilage samples, samples were treated with Chondroitinase ABC (Ch.ABC; Sigma-Aldrich, St. Louis, MO, USA). Three groups (n = 10 per group) were studied for each CA: (i) control group with no GAG digestion; (ii) 8 hours GAG digestion (8h-group); and (iii) 30 hours GAG digestion (30h-group).

Optiray and Iomeron for morphological assessment

A schematic of the morphological assessment of cartilage using Optiray and Iomeron is shown in Figure 2.

Incubation protocol

For assessment of cartilage morphology, Optiray 350 (Mallinckrodt, St. Louis, MO, USA) and Iomeron 350 (Bracco Imaging, Colleretto Giacosa, TO, Italy) were used, both containing 350 mg/mL organically bound iodine. Different CA dilutions and immersion times were tested to determine the optimal conditions for CECT imaging. For each CA, four concentrations of 70, 105, 140, and 175 mg Iodine/mL were tested by preparing 20, 30, 40, and 50% dilutions of CA in PBS, respectively.

MicroCT scanning and analysis

Three samples (n=3) were scanned for each concentration of CA. For each sample, femoral condyle was pre-scanned with microCT (immersion time = 0 minutes), then immersed in 2 mL of CA solution at 37°C for cumulative times of 5, 10, 15, 30 and 60 minutes (total 6 scans for each sample). Following each immersion, the femoral condyle was pat dry and scanned.

Samples were scanned in air using microCT (μCT50, Scanco Medical, Bruttisellen, Switzerland). Projections were acquired at a voxel size of 12 μm, 55 kVp, 57 μA, 0.5 mm Al filter, high

resolution, and 200 ms integration time. The femur was secured in the scanning tube to ensure the longitudinal axis was positioned vertically. To prevent dehydration, the scanning tube contained a small amount of PBS at the base, and the tube was sealed with parafilm.

For a set of scans of each femoral condyle, a cartilage mask volume was manually contoured and then registered to the other 5 scans to ensure they all have a consistent volume of interest. This approach was chosen over a threshold segmentation approach to ensure low attenuating data was not excluded from the analysis, and the boundary between cartilage and bone was consistent independent of CA concentration. Mean CECT attenuation and standard deviation of the mask volume for each sample was calculated for each time point and concentration. Mean CECT attenuation and standard deviation are calculated as the average greyscale value (out of total 32767) of all voxels in the mask volume using the *histogram* function in Scanco Medical's image processing software (IPL v5.42).

Assessment of the correlation between CECT Attenuation and GAG content

A schematic of the preparation and analysis of correlation between CECT Attenuation and GAG content is shown in Figure 3.

GAG digestion

Chondroitinase ABC (Ch.ABC), a GAG specific hydrolase, was used for enzymatic digestion of bovine cartilage GAGs to small oligosaccharides to create a spectrum of GAG content. (Suzuki et al., 1968, Otsuki et al., 2008) Each sample was immersed in 3 mL Ch.ABC digestion solution (0.1 U/mL in 50 mM Trisma-base, 60 mM NaOAc, 0.02% BSA, pH 8.0) at 37°C for a specified digestion time (8 or 30 hours). After 8 hours, the samples (8h-group) were pat dry and stored at -80°C. After 30 hours, samples from both 8h- and 30h-groups were washed with 10 mL containing 1% Antibiotic Antimycotic (A/A) (Sigma-Aldrich, St. Louis, MO, USA) in ultrapure water for 24 hours at 4°C, 750 RPM. After washing, samples were stored at 4°C in fresh 1% A/A solution.

MicroCT scanning and analysis

Following GAG digestion, smaller cores were punched from each sample to obtain Ø3 mm cores with matching Ø5 mm outer rings. The inner core was immersed in CA as described below (where n = 5/concentration/digestion time), whilst the outer ring was used to determine GAG content.

(Nimeskern et al., 2016) For CECT imaging, Iomeron 350 and Optiray 350 solutions were prepared in concentration ratios of 30% and 20% in PBS, respectively, to ensure rapid equilibration.

The inner core of each sample was immersed in the CA for 5 minutes at room temperature. MicroCT scans were performed at a voxel size of 4 μm, 70 kVp, 57 μA, 0.5 mm Al filter, high resolution, and 300 ms integration time.

After scanning, images for each sample were segmented by thresholding the cartilage from the background using a grey-scale attenuation of 100 HU. Post-processing was conducted by creating a solid mask of the cartilage volume after thresholding, from which mean and standard deviation grey-scale attenuation in the sample was calculated.

GAG content assay

The wet weight of the outer ring of each sample was measured prior to lyophilisation for 24 hours. All remaining GAG was extracted with 1 mg/mL papain enzyme (Sigma-Aldrich, P4762, St. Louis, MO, USA) in papain buffer (20 mM sodium phosphate, 5 mM EDTA, 2 mM DTT, pH 6.8) at 60°C for 8 hours. GAG content of each sample was assessed in two replicates, using 1,9-dimethylmethylene blue (DMMB) (Sigma-Aldrich, 341088, St. Louis, MO, USA) colorimetric assay. A microplate reader (Thermo Fisher Multiskan FC, Vantaa, Finland) was used to conduct UV-Visible Spectroscopy, by measuring absorbance of each sample in triplicate at 520 nm. Chondroitin-4-sulfate (Sigma-Aldrich, C4384, St. Louis, MO, USA) from shark cartilage was used to produce a standard curve plotting GAG content against absorbance, with concentrations ranging from 1 to 10 μg/mL of GAG dissolved in papain buffer.

Statistical analysis

To determine optimal immersion time and concentration, CECT attenuation at 60-minutes immersion was considered the equilibrium state, while significantly different CECT attenuation to bone and no-contrast cartilage were considered required for good segmentation. A two-factor analysis of variance was performed to test for significance between immersion timepoints and concentrations, as well as any interaction between them. Post hoc comparisons were performed using Bonferroni's test, or in the case of unequal variances, Tamhane's T2 test. Levene's test of equality was used to determine the error variance. In this way, the earliest equilibrium time was

determined for each CA concentration, and the optimal concentration to delineate the cartilage from other structures (i.e. bone and no-contrast cartilage P-values < 0.05 were considered statistically significant.)

To investigate the correlation between GAG content and CECT attenuation, F-tests for equality of variances were executed to evaluate if the variances differed between each group that was measured in triplicate by UV-Visible spectroscopy. Following this, a t-test with equal or unequal variances, dependent on the F-test outcome, was used to compare the two replicates from each sample to identify significant differences. Significant difference was defined as $p < 0.01$. A 99% confidence interval was chosen since assays groups were taken from the same sample and no significant difference should be present. The relationship between CECT attenuation and GAG content was evaluated by linear regression analysis, $p < 0.05$. Statistical analysis was performed with SPSS Statistics (v24, IBM, NY, USA).

Results

Optiray for morphological assessment

The optimal immersion time for each concentration of CA was defined as the earliest scan-time showing no significant difference in CECT attenuation compared to the 60-minute immersion scan. Figure 4a shows the change in CECT attenuation over time using four concentrations of Optiray 350. No significant difference in attenuation was found from 5 minutes through to 60 minutes for all Optiray 350 concentrations (mean ± SD: 20%: 3423 ± 772 vs 4227 ± 850; 30%: 5266 ± 943 vs 6375 ± 127; 40%: 6101 ± 1882 vs 7321 ± 846; and 7659 ± 564 vs 8434 ± 353, for 5 vs 60 minutes, respectively). Therefore, samples immersed for these times were considered be at equilibrium for the respective concentrations.

For morphological analysis of cartilage, the cartilage should have an attenuation different from the bone after immersion in CA. To identify the best concentration of CA for morphological assessment, CECT attenuation at the earliest equilibrium time for each concentration of CA was compared to that of subchondral bone and no-contrast cartilage, using the average attenuation of bone (22.4%, 7340 units) and no-contrast cartilage (7%, 2294 units) (Figure 4b). Attenuation of

contrast-enhanced cartilage was found to be significantly different ($p < 0.05$) to both no-contrast cartilage and bone for 30% (5-min) concentration (mean ± SD: 5266 ± 943).

Iomeron for morphological assessment

Figure 4c shows the change in cartilage attenuation over time using the four concentrations of Iomeron 350. There was also no significant difference between 5 minutes through to 60 minutes for all Iomeron concentrations (mean ± SD: 20%: 3656 ± 2474 vs 4211 ± 2774; 30%: 4180 ± 966 vs 5991 ± 216; 40%: 6925 ± 811 vs 8831 ± 592; and 8530 ± 346 vs 9423 ± 44, for 5 vs 60 minutes, respectively).

Figure 4d shows the CECT attenuation at the earliest equilibrium time(s) for each concentration compared to bone and no-contrast cartilage. Using Iomeron 350, attenuation of contrast-enhanced cartilage was found to be significantly different ($p < 0.05$) to both no-contrast cartilage and bone for the 30% at 5-min (mean ± SD: 4180 ± 966) and 50% at 5-min (mean ± SD: 8530 ± 346) cases (Figure 4d).

Figure 5 shows representative microCT images of cartilage using 20% (5-min), 30% (5-min), 40% (5-min) and 50% (5-min) Optiray (Figure 5a) and Iomeron 350 (Figure 5b). While Figure 6 shows matching histograms for the yellow insets boxes in Figure 5. In this work, contouring cartilage when using lower contrast agent concentrations (20%, 30%), was found to be easier compared to higher concentrations (40%, 50%). This is supported by the histograms, which – while not bimodal – show more low-attenuating signal (<7340 units) that corresponds to the contrast signal in the cartilage.

Correlation between CECT attenuation and GAG content

To investigate the correlation between GAG content and CECT attenuation of bovine articular cartilage, linear regression for CECT attenuation using Iomeron (Figure 7a) and Optiray (Figure 7b) versus GAG content were plotted. One sample from the Optiray control group was excluded from the correlation assessment as the t-test showed a significant difference ($p = 0.002$) in GAG content between the triplicate measurements of this sample.

CECT attenuation values for Optiray were in the range of 4200 to 6000 units, while values varied with Iomeron from 4500 to 7000. The regression plot of Optiray measured an R^2 value of 0.0172 and p-value of 0.49, whilst Iomeron gave an R^2 value of 0.0065 and p-value of 0.67. This indicates

that for both CAs, no significant correlation was found between CECT attenuation and GAG content.

Discussion

This study shows that Optiray and Iomeron, two non-ionic LOCMs, increase attenuation of articular cartilage in microCT scans, whilst enabling distinction between cartilage and bone. Both CA concentration and immersion time were shown to affect the quality of cartilage visualisation. Our results showed no correlation between CECT attenuation and GAG content of cartilage using Iomeron and Optiray.

MicroCT imaging can produce high resolution, non-destructive 3D evaluation of articular cartilage morphology. Hexabrix is an ionic LOCM contrast, used for preclinical CECT imaging and arthrography (Xie et al., 2009). Despite its effectiveness in CECT, Hexabrix was withdrawn from the market by the manufacturer in 2015 (Food and Drug Administration). Alternative non-ionic LOCMs, including Optiray and Iomeron, have the same indications as Hexabrix, making them prime candidates for its replacement in CECT imaging.

To seek a replacement for Hexabrix, we performed CECT using two clinically available non-ionic CAs, Iomeron and Optiray, to evaluate morphology of mouse femoral articular cartilage *ex vivo*.

In preparing scan protocols, it is necessary to define equilibrium time to ensure a consistent uptake of the contrast solution by the cartilage tissue. (Xie et al., 2012, Kotwal et al., 2012) For both Optiray 350 and Iomeron 350, equilibration was achieved from 5 minutes for all concentrations (20-50%) in mouse femoral cartilage. This is similar to equilibration times reported for other CAs used for CECT imaging of cartilage: 6.7 minutes for Hexabrix (Kotwal et al., 2012) and 6.2 minutes for CA4+ in fresh mouse cartilage. (Lakin et al., 2016) These results also highlight that although high concentrations of CA results in higher attenuation of cartilage in scanned images, more time before imaging is not needed to reach equilibrium.

To assess joint morphology, the contrast of the cartilage should be distinctly different to that of adjacent bone tissue. Ideally, this provides a bimodal histogram, allowing for a global threshold to be applied to extract the various tissues for easy segmentation of different tissues. (Kotwal et al., 2012) For the earliest immersion time of each concentration, mean CECT attenuation of contoured cartilage was compared to bone and no-contrast cartilage. Our results showed that cartilage immersed in higher concentrations (40% and 50%) of Optiray 350 and Iomeron 350 had a similar

attenuation to bone (22.4%, 7340 units), suggesting that higher concentrations of these CAs are not suitable for morphological assessment of cartilage. However, 20 and 30% of Optiray 350 and 30% of Iomeron 350 (105 mg Iodine/mL) produced distinctly different contrast to bone, enabling a reliable segmentation and quantification of cartilage and bone in CECT images. The iodine content of this concentration (105 mg/mL) is similar to iodine content of the optimal concentration of Hexabrix, 128 (mg I/mL). (Xie et al., 2009) .

Separating the peaks of the histogram is achieved by using different concentrations of the CA solution. However, since there are other soft tissues present in addition to the cartilage which also absorb the contrast agents this makes threshold-based tissue segmentation challenging (Figure 6). In this case, additional manual contouring is often also required to segment cartilage and exclude other soft tissues from assessment (Willett et al., 2016, Kotwal et al., 2012, Lakin et al., 2016). In this work, we found samples immersed in lower concentrations, such as 30% of Iomeron 350 and 20 and 30% of Optiray 350, were easier to segment than higher concentrations (40%, 50%). Based on these data, the optimal concentration and immersion time for morphological assessment of joint using the two CAs is 30% (105 mg Iodine/mL) and 5 minutes for Iomeron 350 and 30% (105 mg Iodine/mL) and 5 minutes for Optiray 350.

Hexabrix (ioxaglate) is an anionic CA that distributes inversely proportional to the negatively charged GAGs in ECM. Although ionic CAs can be used for measuring GAG loss in tissues, due to the electrostatic repulsion with GAGs, there is a low magnitude of attenuation in images. On the other hand, cationic CAs bind proportionally to the negatively charged GAGs. However, their strong electrostatic binding increases the time it takes for the body to clear the CA, and results in increased risk of possible adverse reactions. In microCT, attenuation intensities of ionic CAs, such as Hexabrix, (Xie et al., 2010) correlate with cartilage GAG content and that can be used as an indication of the development of OA at early stages. The second aim of this work was to determine whether a correlation exists between CECT attenuation of Optiray and Iomeron and cartilage GAG content. Since Optiray and Iomeron are uncharged, it was hypothesized that no qualitative relationship would be present between CECT attenuation and the GAG content. The concentration and immersion time for both CAs were chosen based on the morphological assessment study, with adjustments in immersion time to offset the absence of subchondral bone, and solution concentration to target an attenuation value of approximately 4000 HU – slightly lower than the midpoint of no-contrast cartilage and bone CT attenuation in Figure 4. Our results supported our

hypothesis, showing that unlike Hexabrix and CA4+ (Bansal et al., 2010) (Xie et al., 2010, Palmer et al., 2006, Nickmanesh et al., 2018, Yoo et al., 2011, Cockman et al., 2006, Kallioniemi et al., 2007), there is no correlations between Optiray and Iomeron CECT attenuation and GAG content of cartilage (Figure 7). This indicates that although Optiray and Iomeron are promising agents for morphological assessment of cartilage, they cannot be used as indicators of GAG-loss related to OA.

Achieving diversity of GAG content was limited by the inherent characteristics of cartilage located at different parts of the bovine femoral head. Although randomly selected, in the Optiray correlation study it was observed that nine of the ten samples of the 30-hour GAG digested group were totally GAG depleted. In contrast, in the Iomeron study only three of the ten samples were totally GAG depleted. Chondroitinase ABC does not display a linear enzymatic activity, so optimising digestion protocols to achieve more diversity in GAG content would be challenging. However, even so, it is unlikely to change the finding that there is no significant correlation between GAG content and CECT attenuation.

It can be observed in Figures 4 and 7, that Iomeron appears to have more variability in CECT attenuation than Optiray. Further investigation of this phenomenon was outside the scope of this study. However, most contrast agents of this kind are defined by their surface charge (and size). Since both Optiray and Iomeron are non-ionic and do not have electrostatic interactions with cartilage components, they may have other chemical interactions where they bind tighter or are trapped within the tissue in Optiray and not Iomeron. This requires further investigation.

Conclusion

In this work, optimal concentration and immersion times for Optiray and Iomeron were identified to provide high resolution morphology of femoral articular cartilage of the mouse by microCT. Although these CAs show comparable result to the discontinued Hexabrix, no relationship was observed between CECT attenuation and GAG content. This suggests that although Optiray and Iomeron can provide valuable morphological data of cartilage, other CAs are needed to replace discontinued agent, Hexabrix, for detecting GAG loss with OA at early stages using CECT.

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Author Contributions Statement:

All authors designed the experiments, analysed and interpreted the data. BVT, CTV and NRYK performed the experiments. CTV, NRYK, NA and KSS wrote the manuscript, and BVT critically reviewed the manuscript. KSS designed the study and provided resources for all experiments. All authors have read and approved the final submitted manuscript.

Data Availability Statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure Legends

Figure 1. Chemical structure of the contrast agent compounds (a) Optiray and (b) Iomeron. (National Center for Biotechnology Information, National Center for Biotechnology Information)

Figure 2. An overview of the sample preparation, scan timeline, and data processing and analysis tasks for assessing cartilage morphology using Optiray and Iomeron.

Figure 3. An overview of the sample preparation, DMMB assay, and microCT scanning and analysis tasks for assessing GAG correlation with CECT attenuation for Optiray and Iomeron.

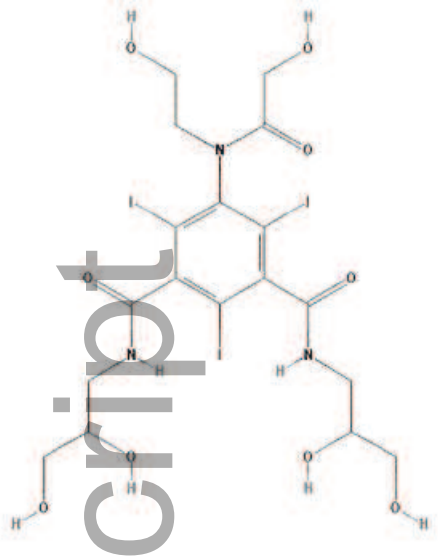
Figure 4. Mean CECT attenuation against time for each concentration of (a) Optiray 350 and (c) Iomeron 350. Mean CECT attenuation for the earliest equilibrium time(s) for each (b) Optiray and (d) Iomeron concentration, compared to bone (solid) and no-contrast cartilage (dashed). * and †

indicate significant difference to bone and no-contrast cartilage attenuation values, respectively ($p < 0.05$).

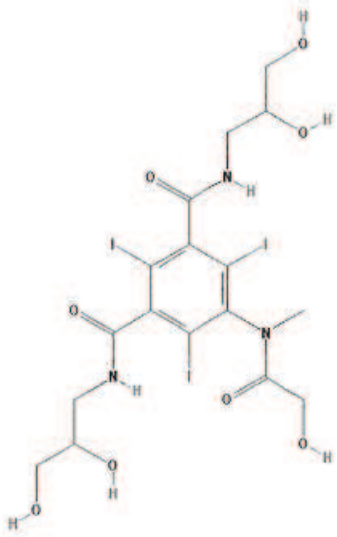
Figure 5. Representative CECT images of mouse cartilage using 20%, 30%, 40% and 50% of (a) Optiray 350, and (b) Iomeron 350. Dashed region of interest insets are used to create histograms in Figure 6.

Figure 6. Histograms of mouse cartilage using 20%, 30%, 40% and 50% of (a) Optiray 350, and (b) Iomeron 350 from dashed region of interest insets in Figure 5.

Figure 7. Linear regression plots of CECT attenuation using (a) Optiray and (b) Iomeron vs. GAG content of bovine articular cartilage. The light blue shaded curve shows the 95% confidence intervals for individual points and the dark blue shaded curve shows the 95% confidence interval curve for the mean.



Optiray (loversol)



Iomeron (lomeprol)

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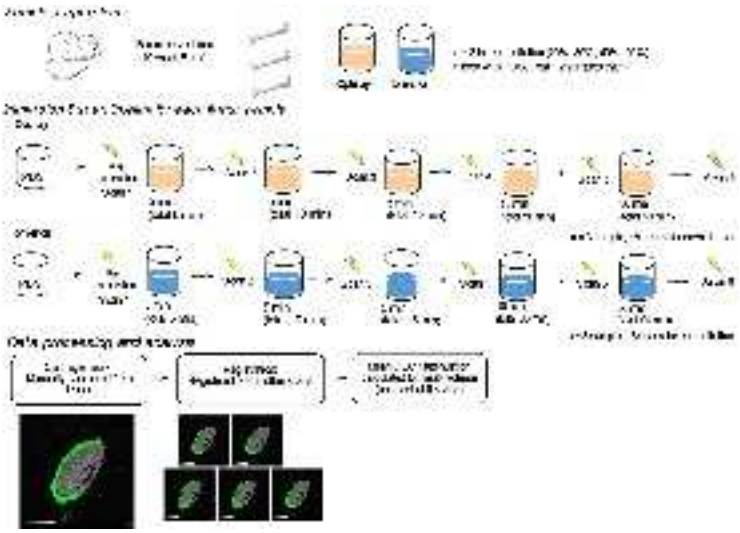

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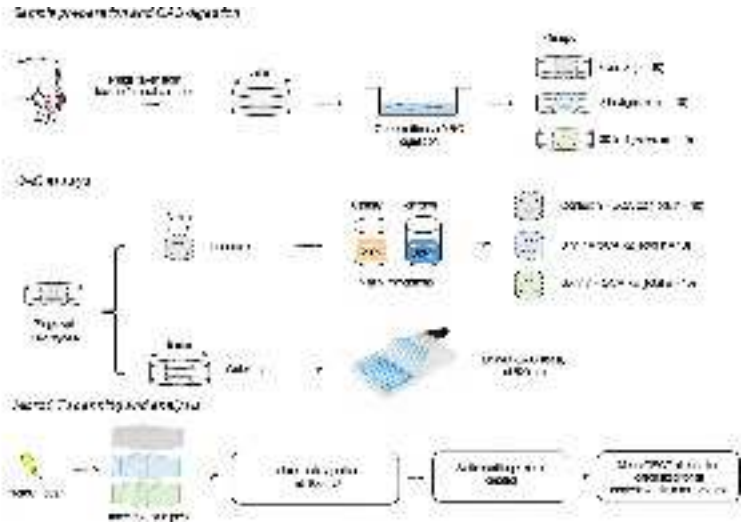

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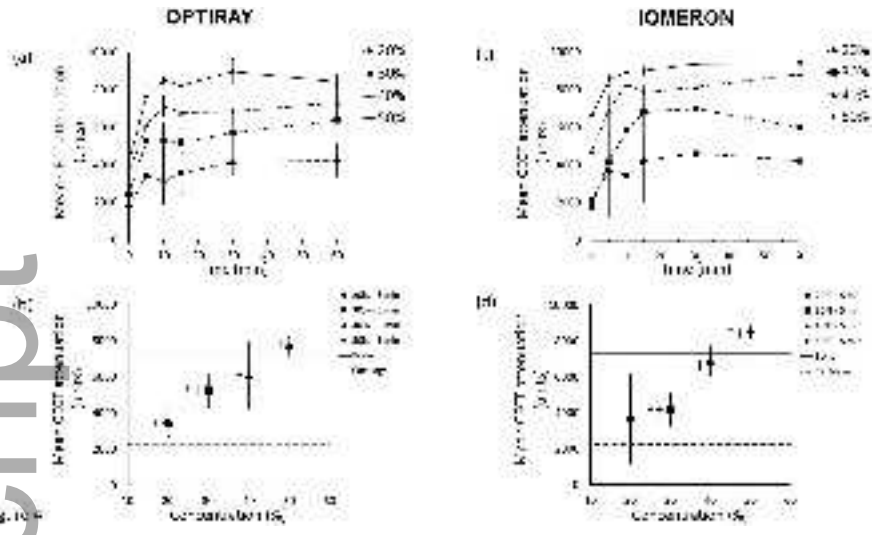

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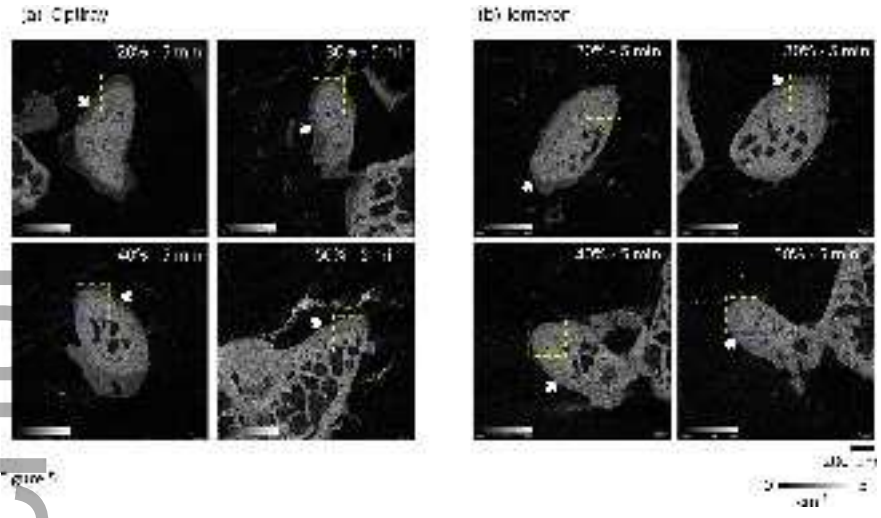

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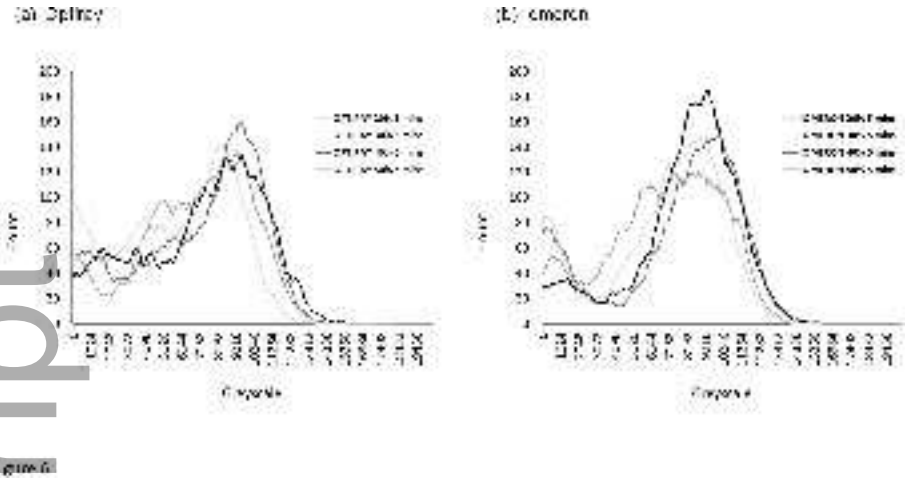

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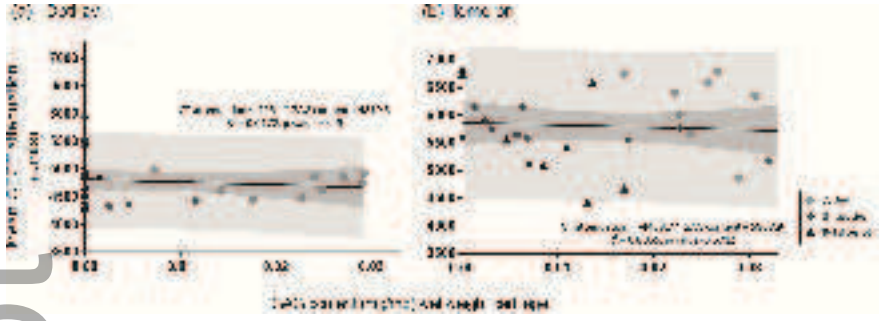

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