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Title: photoacoustic imaging of synovial tissue hypoxia in experimental post-traumatic osteoarthritis

Running title: Hypoxia imaging in OA

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Abstract

Objectives: This pilot study aimed to investigate the feasibility of non-invasively assessing synovial tissue hypoxia *in vivo* using photoacoustic (PA) imaging in a post-traumatic osteoarthritis model and explore its correlation with OA severity.

Methods: The three-dimensional vasculature structure and oxygenation level of synovial tissues of destabilization of the medial meniscus (DMM)-induced osteoarthritis (OA) mice were longitudinally monitored using PA imaging. Vascular volume/tissue volume (%) and tissue oxygen saturation (sO_2) were validated against results obtained by established Power Doppler (PD) imaging and dynamic changes of inhaled O_2 concentration respectively. PA changes were correlated with the histological grading of cartilage damages.

Results: PA-measurements of vascularity and sO_2 demonstrated a strong correlation with localized blood flow detected by PD imaging (r=0.506, $p<0.001$) and inhaled O_2 concentration. DMM knees exhibited much more vascularity in synovial tissue at 4 months after surgery (median 11.3%, IQR: 10.7-15.5%) than the intact knees at time zero (median:5.1%, IQR:3.8-6.8%, $p<0.001$) as well as the sham-operated knees (median: 4%, IQR: 3.75-5.45%, $p=0.017$). Paradoxically, synovial tissue sO_2 was significantly lower in

DDM knees (median: 37.7%, IQR: 36.4-40.6%) than both the intact (47.1%, IQR: 41.9-49.8% p=0.001) and sham-operated knees (45.1% IQR: 45.1-52.4%, p=0.017). The PA-detected synovial tissue hypoxia correlated with the severity of cartilage loss in DMM mice (*rho*=-0.597, p=0.031).

Conclusion: Here, we demonstrated PA imaging can be implemented for non-invasive imaging of the synovial tissue. Under PA imaging, synovitis in OA was characterized by increased angiogenesis and synovial tissue hypoxia; the latter was associated with the severity of OA.

Key words Osteoarthritis, Synovitis, Vasculature, Power Doppler, Photoacoustic tomography

Introduction

Traditionally, osteoarthritis (OA) is believed to be a non-inflammatory joint disorder caused by wear and tear of articular cartilage under abnormal mechanical loading(Wen et al., 2014). Thanks to advancements in multi-imaging modalities, it is now known that OA is a whole joint disorder, involving not only the degradation of articular cartilage but also deformation of subchondral bone and low-grade inflammation, particularly of the synovial tissue(Mathiessen and Conaghan, 2017). According to a previous MOST study, 66% of OA patients exhibit synovial enhancement on gadolinium-magnetic resonance imaging (MRI)(Guermazi et al., 2011), signifying synovitis is prevalent among OA patients. Such synovial inflammation signals can also be detected in 43% of the patients with previous meniscal injuries and menisectomy prior to the onset of radiographic OA(Scanzello et al., 2011). In addition, MRI-detected effusion and synovitis correlate with pain and increased cartilage loss (Roemer et al., 2011). Given the importance of synovitis in the pathogenesis of OA, imaging of synovitis is of great interests in OA care and research.

Synovitis of arthritic joints is characterized by synovial effusion(Clavel et al., 2008,

55 Conaghan et al., 2006, Kornaat et al., 2006), angiogenesis(Bhat et al., 2015, Clavel et al.,
56 2008, Zinn et al., 1999), hypoxia and reactive oxygen species generation(Biniecka et al.,
57 2016, Biniecka et al., 2011, Ng et al., 2010, Biniecka et al., 2010, Xie et al., 2012). Multiple
58 imaging modalities have been widely studied for in vivo assessments of synovitis particularly
59 in inflammatory arthritis, e.g. MRI(Conaghan et al., 2006, Kornaat et al., 2006), B mode and
60 Power Doppler (PD) ultrasonography(Clavel et al., 2008, Bhat et al., 2015), positron
61 emission tomography (PET)(Zinn et al., 1999, Fuchs et al., 2017), florescence imaging(Xie et
62 al., 2012, Horvath et al., 2016) and *etc.*. Despite having a whole plethora of novel imaging
63 techniques made available for research purposes in the past two decades, the current clinical
64 imaging modalities for synovitis is limited to MRI and ultrasonography that is costly and
65 operator-dependent. With limited reproducibility and sensitivity, this requires considerably
66 more research.
67 MRI and ultrasonography are the current golden standard clinical imaging modalities for
68 synovitis, but they are limited to assessment of synovial effusion and angiogenesis(Conaghan
69 et al., 2006, Kornaat et al., 2006, Bhat et al., 2015, Clavel et al., 2008). For evaluation of
70 synovial hypoxia and reactive oxygen species generation in synovitis, only arthroscopic

71 approach(Biniecka et al., 2016, Biniecka et al., 2011, Ng et al., 2010, Biniecka et al., 2010),
72 or contrast agent labeling techniques(Xie et al., 2012) are available, but these techniques are
73 invasive and expensive.
74
75 Photoacoustic (PA) imaging synergizes the strengths of both optical and sonic imaging – the
76 use of near-infrared laser utilizes the optical absorption properties of the endogenous
77 hemoglobin in blood vessels, thus allow imaging of deeper tissue that ultrasound would be
78 capable of otherwise, while achieving a satisfactory spatial resolution of ultrasound(Wang
79 and Hu, 2012). PA imaging has been applied for *in-vivo* non-invasive measurement of tumor
80 angiogenesis, intracranial cortex vasculature and tissue oxygen level of the brain without
81 requiring any contrast agent(Toi et al., 2017, Tang et al., 2015). Technically, it is also feasible
82 to image the structural changes in the small finger joints with OA *in-vivo(Sun et al., 2011,*
83 *Xiao et al., 2010, Xiao and He, 2010)*. Studies have demonstrated the feasibility of PD
84 imaging for evaluating synovitis and vascular invasion in small animals arthritis model(Xu et
85 al., 2017, Clavel et al., 2008), from which the authors observed significant increase in PD
86 signals in posttraumatic OA and RA respectively. The caveat is that PD imaging can only

87 indicate the volume flow rate of blood rather than the actual volume of blood within the
88 tissue, which requires the use of other imaging modalities, prompting us to look into PA
89 imaging.

90 In this pilot study, we aimed to investigate the use of PA imaging as a non-invasive
91 in-vivo evaluation tool for 3-D quantitative measurement of synovial tissue vascularity and
92 hypoxic status in live animals. Then we employed PA imaging to delineate the temporal
93 changes of synovitis with the progression of OA, and to explore its relationship with the
94 severity of articular cartilage damages in a destabilization of the medial meniscus
95 (DMM)-induced OA mouse model.

Methods

Study Design

The institutional Research Ethics Committee approved all the experimental procedures in this study (15-16/17-BME-R-HMRF).

In this study, we first performed destabilization of medial meniscus (DMM) surgery on left hind limb of 6-month-old male BALB/c mice (n=13) and capsulotomy as sham operation on three mice (n=3). The DMM joints were scanned using high-frequency ultrasound (US), PD and PA micro-imaging at 1 month (n=7) and 4 months (n=6) post-surgery, respectively. In order to validate our PA findings, PD and PA imaging was also performed on mice with intact knees as controls **(n=48)**.

Animal surgery

DMM surgery was performed on mouse knees according to an established protocol(Glasson et al., 2007). Briefly, the right hind leg of the mouse was shaved with hair shaving cream and disinfected with iodine solution under general anesthesia via intraperitoneal injection of a ketamine (100mg/ml), xylazine (20mg/ml) and saline (1.0ml:0.5ml:8.5ml, 1ml/100g) cocktail. A longitudinal parapatellar incision was made to open the joint capsule and to expose the

medial meniscus. Subsequently, transection of the medial meniscus was conducted using #11 blade or micro-surgical scissors. The sites were irrigated and the joint capsule, subcutaneous membrane and skin were sutured layer by layer. The mice were allowed to move freely immediately after the surgery.

High-frequency ultrasound, Power Doppler and photoacoustic micro-imaging

Ultrasound imaging was conducted on the live animals using Vevo2100 high-frequency Micro-Imaging System (VisualSonics, Toronto, Ontario, Canada). General anesthesia was maintained with 1%-3% isoflurane inhalation throughout the entire procedure. The right hind limb was first shaved, then the animal was positioned supine onto a heating pad with the extremities attached to the electrode for monitoring the heart rate and respiratory rate. The ultrasound transducer was attached to a 3D motorized articulated arm (MP-Tec AG, Veltheim, Switzerland) and positioned for sagittal views of the mouse knee [**Fig. 1(A)**]. The right leg was flexed at approximately 120 degrees, and coupling gel was applied to allow clear visualization of the knee joint [**Fig. 1(B) and (C)**].

For **B mode and PD imaging**, an ultrasound transducer with a center frequency of 50MHz and a bandwidth of 30-70 MHz (MS700, image axial resolution 30μm) was employed. B

mode imaging acquisition parameters were set as follows: frequency: 50MHz, power: 100%, gain: 35 dB, focus depth: 4.5 mm, focal zone: 3.75-5.25 mm, Line density: full, Persistence: off, Sensitivity: high, Dynamic range: 65dB, Display map: G5. PD imaging settings were defined as follows: Frequency: 40MHz, Power: 100%, PRF (pulse repetition frequency): 1 kHz, Doppler gain: 35 dB, 2D Gain: 35dB, Focus depth: 4.5 mm, Beam angle: 0 deg, Sensitivity: 5, Line density: full, Persistence: low, Dynamic range: 60 dB, Wall filter: high, Priority: 58 %. For **PA imaging**, a transducer with a center frequency of 30MHz and a bandwidth of 18-38MHz (LZ400, image axial resolution 50um) was adopted. The raw PA signal intensity was measured at two wavelengths: 750 nm (for deoxygenated hemoglobin) and 850 nm (for oxygenated hemoglobin)(Wilson et al., 2014). The PA imaging settings were defined as follows: Frequency: 30MHz, Power: 100%, 2D gain: 20 dB, PA gain: 46 dB, Focus depth: 6 mm, Line density: high, Persistence: off, PA acquisition: sO2/HbT, Priority: 99%, Threshold HbT: 20. Since the cardiac and respiratory function and body movement would largely affect the PD and PA signals, heart rate & respiratory rate were closely monitored after inhaled anesthesia and during PD and PA image acquisition.

Three-dimensional scan of entire mouse knee joints was conducted by linear translocation

144 of the transducer perpendicular to the single sagittal plane of 2D imaging. The total distance
145 of 3D scan was set to 5mm to cover the entire joint with a step size of 0.032mm. A triangular
146 region of interest (ROI) was manually drawn in the frame that best featured the triangle
147 defined by the patella tendon (the dark hypo-echogenic line parallel to the skin surface),
148 proximal tibia and distal femur (tendon-tibia-femur triangle, TTF) in the sagittal view of
149 mouse knee joint as previously described [**Fig.1(D)-(F)**](Clavel et al., 2008). The height of
150 ROI was set from the lowest point of the TTF to 0.3mm above the highest surface of the skin,
151 and the length was set to the length of the patella tendon, linking the highest point of the
152 femur and tibia in the image. During 3D data analysis, the same ROI was segregated
153 automatically in all the 2D slices by a custom-designed script on Matlab (Vessel analysis
154 v1.4)(Sun et al., 2012, Cheung et al., 2012). The vascular volume (%) was quantified by
155 counting colored pixels at the resolution of 0.010 mm×0.010 mm in the ROI on each PD and
156 PA slice, then multiplied by 0.032 mm (slice thickness), and then divided by the total number
157 of voxels in the volume of interest (VOI). Similarly, the average sO_2 (%) was converted from
158 the mean PA signal intensity of the voxels in the VOI.
159 **For PA imaging,** we collected 1000 consecutive PA frames with the inhaled gas switched

160 from air (O_2 concentration: 21%) to 100% pure oxygen. The sO_2 graph of a selected blood
161 vessel was plotted to observe the time-dependent change of sO2 with different concentrations
162 of inhaled O_2 (**Supplementary Fig.1.**)

163 **μCT-based micro-angiography**

164 The μCT-based micro-angiography was performed on 2 animals per group following an
165 established protocol(Zhen et al., 2013). In brief, the mouse heart was gently exposed under
166 deep anesthesia. A 30-gauge needle was inserted into the left ventricle for perfusion and a
167 small incision was performed to the right atrium for drainage. The blood was washed away
168 from the vessels using 0.9% normal saline solution containing heparin sodium (100U/ml),
169 followed by 10% neutral buffered formalin for blood vessel fixation. After the removal of
170 formalin by repetitive infusion of saline, the vascular system was perfused with Microfil
171 MV-117 (Flow Tech, Carver, MA). The samples were stored at 4°C overnight to allow
172 polymerization of Microfil. The knee joints were fixed in neutral buffered formalin for 48
173 hours and decalcified in 10% EDTA (pH 7.4) for 20 days. The specimens were scanned via
174 VivaCT 40 (Scanco Medical, Bruttisellen, Switzerland) at 10.5μm voxel size.
175 Three-dimensional vasculature images in the previously defined triangle ROIs were

reconstructed for comparison with PA images.

Histology

Histomorphological evaluation of OA was performed on the baseline control (n=4), 1-month post-surgery (n=7) and, 4-months post-surgery (n=6) animals. Fixed and decalcified samples were dehydrated in alcohol gradient and embedded in paraffin. Five-micron-thick sagittal serial sections of knee joint medial compartment were made for hematoxylin and eosin (H&E) staining. Images were captured using OLYMPUS BX51WI Microscope and Image-Pro Plus version 5.0 (Media Cybernetics, Inc.). Neovascularization of the knee joint was evaluated; the severity of OA including articular cartilage damage (0: intact; 1: surface fibrillation; 2: vertical fissure; 3: cartilage worn-out; 4: cartilage worn-out down to subchondral bone), synovitis (0: intact: 1: high cellularity; 2: high cellularity and vascularity), osteophyte (0: absent; 1: present) and meniscus ossification (0: absent; 1: present) was graded using an OARSI recommended histological grading system after minor modification(Kamekura et al., 2005, Glasson et al., 2010). Two independent observers graded three equidistant sections at 60-70μm apart throughout the medial compartment of the knee joint in a blinded manner. The average score was taken.

192 **Statistics**

193 All data were presented in median (interquartile range). The relationship between PA and
194 PD data in the intact mice were analyzed using Pearson or Spearman's correlation. The
195 comparisons of PA and PD data of DMM mice at different time points were performed using
196 Kruskal–Wallis H test; when the overall statistical significance was indicated, *post-hoc* Mann
197 Whitney U test was performed. The links of the ultrasound, PD and PA imaging findings of
198 synovitis with histopathological grading of articular cartilage damages were explored using
199 Spearman's correlations. The level of significance was set at 0.05 (IBM SPSS statistics v.21,
200 U.S.A.).

Results

Validation of PA-based synovial tissue vascularity and oxygen saturation in intact knees

In intact mouse knee joints, stable PD and PA signals were present subcutaneously, consistently localized around patella tendon and joint capsule (**Fig.1&2**). Median of PA-based vascularity volume/tissue volume (PAVV) was median 5.1% (IQR: 3.8-6.8%) whereas PD-measured vascularity volume/tissue volume (PDVV) was only 1.7% (IQR: 1.2-2.1%) at the baseline in the intact knees. It was found that PAVV was much larger than PDVV ($p<0.001$, with paired *t* test). Moreover, PAVV moderately correlated with PDVV ($r=0.506$, $p<0.001$) (**Supplementary Fig. 2.**).

Meanwhile, synovial tissue oxygen saturation (sO_2) generated from PA images responded sensitively to the alterations of the different concentrations of inhaled O_2 in intact mice (**Supplementary Fig.1.**). The average subcutaneous tissue oxygenation level reached around 90% when the animals received pure oxygen. In contrast, the synovial tissue was hypoxic and sO_2 was 47.1% (IQR: 41.9-49.8%) in intact knees. There was a weak but statically significant association between synovial tissue sO_2 and PAVV ($r=-0.307$, $p=0.010$), no association was found between synovial tissue sO_2 and PDVV ($r=-0.031$, $p=0.803$).

Temporal changes of PA findings of synovitis in DMM knees (Fig.2&3)

Compared to intact knees, PDVV did not significantly change at 1-month (2.5%, IQR: 1.5-3%) yet dropped at 4-month post-surgery (1.1%, IQR: 1.1-1.2%). PDVV of the sham-operated knees at 1-month (2.8%, IQR: 2-3.2%) and 4-month post-surgery (1.9%, IQR: 1.75-2.2%) was not significantly different from the baseline. PDVV of DMM knees was lower than sham-operated ones at 4 months (p=0.014) yet not at 1-month post-surgery (p=0.918).

Compared to intact knees, PAVV in DMM knee dramatically increased at 1- (10%, IQR: 8.25-23.73%, p=0.001) and 4-month post-surgery (11.3%, IQR: 10.65-15.5%, p<0.001) (Fig.7). PAVV of the sham-operated knees at 1-month (9.5%, IQR:7.6-9.8%, p=0.014) and 4-month post-surgery (4%, IQR: 3.75-5.45%, p=0.603) did not differ from the intact knees. In addition, PAVV in DMM knees did not differ from those of sham-operated knees at 1 month after surgery (p=0.474), but it was significantly higher at 4-month postoperatively in DMM knees than in the sham-operated ones (p=0.17).

Similarly, synovial tissue oxygenation level, sO_2, was much lower in DMM knees at 4-month (37.7%, IQR: 36.4-40.6%) than at 1-month post-surgery (42.6±1.7%, 38.6~46.6%)

(42.1%, IQR: 38.7-46.3%) as well as the intact knees (p=0.005). Synovial tissue sO_2 did not change with healing over time in sham-operated ones (1-month: 46.8%, IQR: 45.1-50.7%, 4-month: 45.1%, IQR: 45.1-52.4%). Most interestingly, the level of sO_2 in synovial tissues was closely associated with the PD-detected blood flow (Spearman's Rho=0.733, p=0.004).

PA imaging of synovitis was associated with articular cartilage damages in DMM knees

As shown in the histopathological images of DMM knees, synovitis was characterized by hypercellularity and neoangiogenesis at 1-month after surgery(**Fig.4.**). Blood vessel of smaller diameter and leakage of RBCs to interstitial space were observed in synovial tissues of the 4-month post-surgery group. It was accompanied by vascular invasion into injured meniscus with ossification formation. The histological findings were compatible with the temporal changes of PAVV and PDVV. In addition, the PA image of microvasculature inside joint was close to the 3-D reconstructed vasculature under μCT-based micro-angiography in our region of interest (**Supplementary Fig.3.**).

The synovial tissue sO_2 level was negatively associated with the severity of articular cartilage (Spearman's Rho=-0.597, p=0.031) and entire OA joint destruction (Spearman's Rho=-0.555, p=0.049)(**Fig.5.**). In addition, synovial tissue hypoxia was also associated with

249 osteophyte formation (Spearman's *Rho*=-0.592, p=0.033), and correlated with meniscus

250 ossification with marginal statistical significance (Spearman's *Rho*=-0.507, p=0.077).

251 Actually, PDVV also showed marginal statistically significant correlations with meniscus

252 ossification and overall OA joint damages.

Discussion

There are a few major limitations to be addressed before any conclusion can be drawn from this pilot study. First, the current study is limited by the cross-sectional design meant for validating PA findings by means of histopathology. Thus our findings regarding the correlation between articular cartilage damage and synovial tissue hypoxia cannot unveil any causal relationship. Second, since the focus of the present study was placed on PD and PA images, we did not quantify the non-specific changes of gray scale in B mode ultrasound image with the progression of OA. These signs may potentially indicate synovial effusion and edema. Third, it was not feasible to differentiate soft tissues, such as synovium, meniscus and articular cartilage, insides joint capsule in micro-ultrasound images of knee joint. Thus we defined a rather large ROI to cover all affected joint tissues. Considering the nature of these tissues, the vasculature was predominantly present in synovium instead of meniscus and articular cartilage. PA and PD changes mainly reflected synovial inflammation.

This study employed the cutting-edge PA imaging modality for *in-vivo* measurement of synovitis. We for the first time reported synovial tissue hypoxia along with vasoconstriction (suggested by PD imaging and histopathological findings) and with deterioration of articular

269 cartilage damage in a well-received DMM mouse model. Use of three-dimensional PA and
270 PD imaging analyses approach in this study minimized the bias potentially brought by the
271 operator-dependent imaging probe position and sagittal plane selection in the scanning. In
272 addition, we made one step forward by introducing a quantitative analysis protocol for the PD
273 and PA imaging of synovitis compared to the semi-quantitative grading system as previously
274 reported in both human and animal models(Clavel et al., 2008, Atukorala et al., 2016).
275 Both PA and PD are effective in visualising blood vessels inside biological tissues, but
276 their imaging principles are different. Intensity of PA signals depend on the amount of the
277 hemoglobin of red blood cells (RBCs) in the blood stream. While PD signals rely on the flow
278 rate of blood and RBCs inside blood vessels, which are not only affected by the number and
279 size of blood vessels but also their perfusion and permeability function. Therefore, the PD
280 and PA findings are intrinsically linked as indicated by the correlative analysis in the present
281 study. However, in this study, we observed that PA signals were consistently stronger than PD
282 signals in intact and DMM knees. We suspect this phenomenon can be attributed to the fact
283 that newly formed blood vessels with synovial angiogenesis contain hemoglobin but might
284 not be well connected to the pre-existing vasculature, as shown in previous study(Zhen et al.,

285 2013). As a result, the low velocity blood flow in the newly formed vessels could be easily
286 detected by PA images but may go undetected in PD images. In the injured knees after DMM
287 surgery, we have showed that PA imaging could detect synovial neoangiogenesis at 1-month
288 and vascular invasion into meniscus at 4-month post injury. In contrast, PD imaging, the
289 current standard clinical assessments of synovitis, mainly reflected the functional changes of
290 vasculature in synovium with healing over time in our DMM mouse model. The volume of
291 PA signals reflected the amount of both pre-existing and newly formed blood vessels, while
292 PD signals could reflect the size and function of the existing vasculature. In our study, the
293 decrease of PD signals but increase of PA signals occurred at 4-month post DMM surgery.
294 This indicates reduction of the size of blood vessel, e.g. the vasoconstriction, and increased
295 vascular permeability with RBCs leakage to interstitial space in the advanced stage of
296 experimental OA. This finding is in good agreement with MRI-detected impaired vascular
297 permeability function in a posttraumatic OA mouse model(Zhen et al., 2013). High level of
298 plasma and synovial endothelin-1, a potent vasoconstrictor, in the advanced stage of knee OA,
299 might be the molecular basis for this phenomenon(Zhao et al., 2016, Sin et al., 2015). As
300 such, it may partially explain why synovial tissue sO_2 was associated with PD-detected blood

vessel function in DMM knees. Taken together, PD and PA are complementary to each other in providing comprehensive information about vascular structure and function in healthy and diseased conditions.

The image pattern of synovitis we observed in DMM-induced OA mouse model differs from a previous report using comparable high-frequency ultrasonography and PD imaging in collagen-induced arthritis (CIA) mouse model in a way that PD-detected signal changes in CIA model were present after CIA induction, and they correlated with the histopathological severity of synovitis(Clavel et al., 2008). Compared to the sham-operated knees, the significant changes in the PD and PA images of DMM knees were present at 4-month after surgery instead of 1-month after surgery. It implied that DMM contributed to synovial tissue vasoconstriction, hypoxia and vascular invasion whereas capsulotomy – sham operation itself led to the increase of angiogenesis. With the sham-operated mice at the same age as the DMM mice, we could exclude growth between the operation and the endpoint as a confounding factor in the readout of PD and PA images. To confirm this, we also looked into 6 to 10-month-old mice and did not find any age-related changes in PD and PA images (*unpublished data*). In addition, it was noted that the wall filter was set at a relatively lower

level, i.e. 2.5mm/s, in the previous study(Clavel et al., 2008). As a consequence, it would inevitably bring obvious background noise in PD images. In our study, we chose high wall filter in order to achieve high reliability by sacrificing the sensitivity of PD imaging.

Difference in absorption spectra of oxygenated- and deoxygenated-hemoglobin allows us to probe tissue oxygen level non-invasively under PA imaging. Alterations of oxygen tension such as hypoxia are involved in the angiogenesis, inflammation, oxidative damages and apoptosis process during OA pathogenesis (Conaghan et al., 2005, Findlay, 2007). One case report of hand OA showed PA imaging of low tissue oxygenation level in the affected finger joints(Sun et al., 2011). In this study, we systemically explored the diagnostic values of PA imaging for OA. We found PA-detected synovial hypoxia occurred at the advanced stage of experimental OA, and positively correlated with the severity of cartilage damage and joint destruction. Clinically, invasive arthroscopic approach using Licox probe is the gold standard measure for synovial tissue oxygenation level (Biniecka et al., 2016, Biniecka et al., 2011, Ng et al., 2010, Biniecka et al., 2010). Our study suggested a great potential of PA imaging in clinical practice as an alternative approach to measure synovial hypoxia and to detect arthropathy in a non-invasive manner.

According to PD and PA findings from the present study, the development of OA could be divided into compensatory and de-compensatory stage. In the compensatory stage, the synovial tissue is normoxic and has increased angiogenesis. With the progression of disease, it enters the de-compensatory stage with vasoconstriction and synovial tissue hypoxia, which has been proven to correlate with various arthropathies including menisci ossification, osteophytosis, and also articular cartilage damages. In other words, PD and PA imaging of synovial tissue vasoconstriction and hypoxia are potential biomarker candidates for progressive and non-progressive OA. In contrast, we failed to detect any association between PA findings of angiogenesis with the severity of OA, also the difference in PA-based vascular volume between DMM and sham-operated group at 1-month after surgery. It pointed in the research direction that vascular function, rather than vascular volume, played a pivotal role in the progression of OA.

In summary, our pilot study demonstrated that PA imaging allows detection of synovial tissue hypoxia in DMM-induced experimental OA. A longitudinal study is much needed to explore whether PA-detected synovial tissue hypoxia is a robust imaging biomarker for the non-progressive and progressive OA.

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Author contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. WEN and Dr. RONG had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design: WEN, RONG, LIU, SUN, Lai and ZHENG.

Acquisition of data: ZY LIU, MT AU, X WANG, and YP ZHENG.

Manuscript revision and data reanalysis: PMB CHAN, L SUN and P Lai

Interpretation of data: all authors.

Competing interests

The authors have declared that no competing interests exist.

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