Joint blood flow is more sensitive to inflammatory arthritis than oxyhemoglobin, deoxyhemoglobin, and oxygen saturation

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Abstract: Joint hypoxia plays a central role in the progression and perpetuation of rheumatoid arthritis (RA). Thus, optical techniques that can measure surrogate markers of hypoxia such as blood flow, oxyhemoglobin, deoxyhemoglobin, and oxygen saturation are being developed to monitor RA. The purpose of the current study was to compare the sensitivity of these physiological parameters to arthritis. Experiments were conducted in a rabbit model of RA and the results revealed that joint blood flow was the most sensitive to arthritis and could detect a statistically significant difference (p<0.05, power = 0.8) between inflamed and healthy joints with a sample size of only four subjects. Considering that this a quantitative technique, the high sensitivity to arthritis suggests that joint perfusion has the potential to become a potent tool for monitoring disease progression and treatment response in RA.

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OCIS codes: (170.3660) Light propagation in tissues; (170.3880) Medical and biological imaging; (170.6510) Spectroscopy, tissue diagnostics.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory arthropathy affecting about 1% of the population [1]. The disease is associated with pain [2,3], reduced quality of life [4], and progressive joint damage that can lead to loss of productivity and profound disability [5–8]. However, recent evidence has shown that the devastating effects of RA can be prevented, or at least significantly reduced, by early diagnostic and adequate treatment of the underlying inflammation [9,10].

RA is typically diagnosed by clinical examination, patient self-assessment, and laboratory tests, which can be supplemented with diagnostic imaging (magnetic resonance imaging, MRI; ultrasonography, US; X-ray radiography) for improved accuracy [11–14]. This multipronged approach has proven to be highly effective in diagnosing RA [12]. Nevertheless, RA treatment remains challenging, despite the advent of potent anti-rheumatic therapies such as biologic drugs [15,16], because of the lack of adequate monitoring tools. The current practice is to use the same tools for both diagnostic and treatment monitoring [17]. This is a major hindrance because, while the aforementioned methods are appropriate for one-time use (e.g., for diagnostic), they are not suitable for longitudinal assessment of joint inflammation in response to treatment. Specifically, clinical examination based on joint counts—the cornerstone of RA treatment monitoring in clinical trials—is too time consuming and as such, is seldom used in clinical practice [18]. As well, the high inter-operator variability of US and the high cost of MRI preclude their wide clinical adaptation [13,19], while laboratory tests and X-ray radiography do not have enough sensitivity to monitor treatment [20]. There is clearly a need for safe, fast, and inexpensive methods that can objectively assess treatment response with a sensitivity that rivals clinical examination and diagnostic imaging.

It is well established that joint hypoxia plays a pivotal role in the progression and perpetuation of RA [21–23]. Since hypoxia affects both tissue blood content and oxygenation [22,24], near-infrared optical methods—diffuse optical spectroscopy (DOS) and tomography (DOT), and photoacoustic tomography (PAT)—that are sensitive to these physiological parameters are being developed to supplement/replace clinical assessment and radiology for RA monitoring [25–34]. These emerging techniques, DOS in particular, have the potential to overcome most of the limitations of current RA monitoring methods since they are safe, relatively inexpensive, and have the potential to quickly and objectively assess RA inflammation at the individual joint level [34,35]. Early applications were limited to measuring joint optical properties at a single wavelength and applying classification algorithms to distinguish inflamed from healthy joints [36–38]. Improved sensitivity was later achieved by measuring joint absorption at multiple wavelengths to estimate oxyhemoglobin (HbO2) and deoxyhemoglobin (Hb) content, and oxygen saturation (StO2) [28,39].

Although several reports have confirmed the capacity of the latter approach to distinguish inflamed from healthy joints (i.e., diagnosis), treatment monitoring will likely require the ability to detect subtler changes than the difference between healthy and arthritic joints. Since hypoxia is also a potent signal for blood flow (BF) [40,41], which is tightly regulated to meet tissue demand, concomitant measurement of perfusion and oxygenation could provide improved sensitivity to joint hypoxia and, consequently, to RA inflammation. Towards the
goal of developing a DOS technique for RA treatment monitoring, the aim of the current study was to compare the sensitivity of BF, HbO₂, Hb, and StO₂ to joint inflammation. Joint BF, Hb, HbO₂, and StO₂ were measured in a rabbit model of inflammatory mono-arthritis before and after induction of arthritis using a multi-wavelength time-resolved DOS system.

2. Methods

2.1 Instrumentation

The light source of the time-resolved DOS (TR-DOS) system consisted of a laser driver (PDL 828, PicoQuant, Germany) and three cooled pulsed diode lasers (LDH Series, PicoQuant, Germany) emitting at 760, 802, and 830 nm. The repetition rate was set to 80 MHz and the power output of each laser was attenuated by two variable neutral density filters (NDC-50-4M, Thorlabs, Newton, NJ) before being coupled into a trifurcated multimode fiber optic bundle (N.A. = 0.22, core 400 µm, 4.7 mm outer diameter; Fiberoptics Technology, Pomfret, CT) which delivered the light to the knee. Light transmitted through the knee joint was collected by a 2-m fiber optic bundle (N.A. = 0.22, core 200 µm, and 3.6 mm active area; FiberTech Optica, Kitchner, ON, Canada) whose distal end was secured in front of an electromechanical shutter (SM05, Thorlabs) coupled to a cooled photomultiplier tube (PMT; PMA hybrid 50, PicoQuant, Germany). The PMT was connected to a time-correlated single photon counting (TCSPC) module (HydraHarp 400, PicoQuant, Germany) to generate temporal-point-spread functions (TPSFs) from the collected photons. The instrument response function (IRF) was also measured using our previously described method [42,43].

2.2 Animal model and experimental procedure

Experiments were conducted on adult male New Zealand white rabbits, using an established animal model of inflammatory mono-arthritis [44,45]. Joint inflammation was induced in the right knee by repeated intra-articular injection of 0.1 mL of a saline solution containing 2% \( \lambda \)-carrageenan at five to seven-day intervals over a four- to six-week period. This model has been shown to produce an inflammatory response similar to human rheumatoid arthritis inflammation [44]. The left knee was injected with 0.1 mL of saline for control. Inflammatory reaction was monitored regularly by visual inspection and palpation.

For each animal two baseline TR-DOS measurements were acquired; one set of data (BF, HbO₂, Hb, and StO₂) were obtained 3-5 days before the first \( \lambda \)-carrageenan injection and an additional set was acquired right before the first injection. Thereafter, five sets of measurements (separated by 5-7 days) were acquired during the inflammation period. Measurements were acquired with the emission and detection probes positioned transversely across the knee joint as shown in Fig. 1. The typical width of the knee-joint was 20 mm at baseline but could increase to up to 25 mm in the inflamed knees. The probes were secured on the knee with help of a probe-holder, which was maintained in place by a strong and flexible support (dark blue in Fig. 1). The probe-holder was positioned on top of the knee cap, which was used as anatomical landmark to ensure repeatability of the positioning of probes. First, three sets of TPSFs, one set for each laser wavelength, were collected on one knee (randomly). The probes were then removed and placed on the other knee and a new set of TPSFs were acquired. These measurements were used to obtain the joint absorption coefficients (at the three laser wavelengths), which were subsequently employed to compute Hb, HbO₂, and StO₂. Following these measurements, two sets of dynamic contrast-enhanced (DCE) measurements were acquired; one set of two measurements per knee. Each DCE measurement involved the injection of the optical contrast agent indocyanine green (ICG) into an ear vein and continuous acquisition of TPSFs with the 802 nm laser for about 250s. The TPSFs were later fit with a theoretical model to estimate the time-dependent concentration of the contrast agent in the knee. Concomitant to the TPSFs measurements, the arterial concentration of ICG was also measured using a dye-densitometer (DDG 2001, Nihon
Kohden, Japan) placed on a front paw. It is worth noting that this approach enables to noninvasively measure the arterial concentration of the tracer without the need for a femoral line. All procedures were carried out while the animals were anesthetized with 3% isoflurane and according to the guidelines of the local ethic committee.

Fig. 1. Positioning of the optical probes on the rabbit knee.

2.3 Data analysis

The TR-DOS measurements were analyzed by fitting the TPSFs with the IRF convolved with a theoretical model of light propagation in tissue:

$$\min_{\mu_a, \mu'_s} (TPSF - IRF \ast \text{TheoreticalModel})$$

(1)

where $\mu_a$ is the absorption coefficient and $\mu'_s$ is the reduced scattering coefficient of the knee. Light propagation in highly scattering and low absorbing media such as tissue can be described using the diffusion approximation to the radiative transfer equation, subject to the measurement geometry and boundary conditions [46]. In the following, we discuss the choice of the measurement geometry and boundary conditions used in the data analysis.

The typical size of a rabbit knee joint was \(~20\text{mm} \times 40\text{mm} \times L\), where $L$ is the length of the leg. Thus, the area of the knee joint was approximated as a parallelepiped of 20 mm width, 40 mm height and length $L$—typically longer than 160 mm, which can be considered optically infinite. Since the DCE-DOS measurements involved acquiring a large number of TPSFs (about 600), we investigated the possibility of analyzing the data using an infinite slab theoretical model to reduce the computational burden [47]. Time-resolved data of a parallelepiped ($20\text{mm} \times 40\text{mm} \times 160\text{mm}$) with linearly increasing $\mu_a$ (0.01-0.08 mm$^{-1}$) and a fixed $\mu'_s$ (1 mm$^{-1}$) were simulated using NIRFAST [48]. The simulated data were analyzed using the infinite slab theoretical model and the recovered optical properties ($\mu_a$ and $\mu'_s$) were compared to the input values to assess the validity of the approach.

For the in vivo data, Hb, HbO$_2$, and StO$_2$ were determined from the three absorption coefficients (one at each laser wavelength) measured before ICG injection. Using the extinction coefficients of oxygenated and deoxygenated hemoglobin at the laser wavelengths, and assuming a water concentration of 70% in the joint, the concentrations of Hb and HbO$_2$ were estimated following the method described in [49], Ijichi et al. StO$_2$ was subsequently computed using the following relation:

$$StO_2 = \frac{HbO_2}{HbO_2 + Hb}$$

(2)
Furthermore, the ICG concentration in the knee was determined in three steps:

1. Each TPSF was fit using Eq. (1), in which the knee joint was modeled as an infinite slab, to obtain $\mu_a$. 

2. The mean absorption coefficient measured during the first 10 s, before injection of ICG, was used as the initial absorption coefficient ($\mu_a^0$) and subsequently subtracted from all $\mu_a$ to obtain the absorption change due to the passage of the bolus of contrast agent through the knee. 

3. The absorption changes were converted into ICG concentration in the knee using the following equation:

$$Q(t) = \frac{[\mu_a(t) - \mu_a^0(t)]}{\ln(10) \times e_{ICG}}$$  

(3)

where $e_{ICG}$ is the extinction coefficient of ICG at 802 nm (i.e., 18.6/mM/mm) [50].

$Q(t)$, the time-dependent ICG concentration in the knee, is related to the arterial concentration of ICG, $C_a(t)$, by the following equation [51]:

$$Q(t) = C_a(t) \times F \cdot R(t)$$  

(4)

where $F$ is the blood flow and $R(t)$ is the impulse residue function, which represents the fraction of dye that remains in the tissue volume at time $t$ following an idealized bolus injection of unit concentration at $t = 0$. Knee blood flow ($F$) was estimated from Eq. (4), using our previously described approach [51].

2.4 Statistical analysis

For each subject, the physiological parameters (i.e., BF, Hb, HbO2 and StO2) measured before the 1st $\lambda$-carrageenan injection were averaged to represent the baseline measurements. Likewise, the measurements acquired after induction of arthritis were averaged to estimate the mean physiological parameters during the post baseline period. Friedman’s Two-way Analysis of Variance (ANOVA) was conducted, using SPSS Statistics 20, to compare measurements acquired on the control (left) and arthritis (right) knees at baseline and after induction of inflammation. The analysis was performed for all the physiological parameters and for those in which the Friedman’s test revealed a significant effect, differences were uncovered using paired-samples $t$-test. All data are presented as mean ± standard error of the mean and statistical significance is based on $p$-value < 0.05.

3. Results

Figure 2(a) shows a plot of the absorption coefficients recovered from the simulated data—by using the infinite slab theoretical model in the fitting—versus the $\mu_a$ used in the simulations. There was a very strong correlation ($R^2 = 0.99$) between the two data sets with a slope of unity and no significant bias. These results indicate that for our transmission measurements, neglecting the finite size of the slab in the directions other than that of the two optical probes has no significant effects on the accuracy of the recovered absorption coefficients (and scattering coefficient as well; data not shown). Consequently, the measurements acquired on the rabbit knee were analyzed using the infinite slab model, which is less computationally expensive. Figure 2(b) displays a TPSF measured on the knee joint and the theoretical curve obtained by convolving the IRF (shown in black) and the infinite slab model. By fitting the measured TPSF with the theoretical curve, the joint optical properties could be estimated. For the example shown in Fig. 2(b), the absorption and reduced scattering coefficients (at 830 nm) were 0.017 and 0.928 mm$^{-1}$, respectively.
Fig. 2. (a) Plot of the $\mu_a$ recovered from the simulation data versus the expected values. (b) TPSF measured at 830 nm on the knee joint and a plot of the theoretical model based on the infinite slab geometry. The instrument response function (IRF) is shown for reference.

In Fig. 3 the ICG concentration in the knee was multiplied by 100 for visualization because the blood in the microvasculature of the knee is a small fraction of the total blood volume. Thus, the concentration of ICG in the knee is much smaller than its concentration in arterial blood. In the example shown in Fig. 3, the knee blood flow was 7 ml/min/100g.

The animal experiments involved four rabbits; each was studied over a period of five to six weeks, which typically yielded seven sets of measurements per subject. Since this animal model involves repeated intra-articular injections, which inherently caused variability in the inflammatory response, the physiological parameters were grouped into “Baseline” and “Post” (i.e., post induction of inflammation). This is to minimize the confounding effects of the natural variability in the inflammatory response due to multiple injections. Table 1 displays the average physiological parameters measured at baseline and after induction of arthritis (Post) in the control (healthy) and inflamed knees. The data show trends towards decreased HbO₂ and StO₂, and increased Hb and BF in the arthritic knees. However, the Friedman’s test revealed that only the differences in blood flow were statistically significant ($p<0.05$); the distributions of the other parameters did not reach statistical significance. We subsequently conducted paired-sample t-tests on the BF data to elucidate the source of the difference detected by the Friedman’s test. The results revealed that there was no difference in blood flow between baseline and post in the control knees. In contrast, the blood flow of the arthritic knees was significantly higher ($p<0.05$) than the perfusion measured at baseline.
Table 1. Blood flow (BF), oxyhemoglobin (HbO2), deoxyhemoglobin (Hb) and oxygen Saturation (StO2) measured at baseline and after induction of arthritis in the control and arthritic knees. Data are presented as mean ± standard error of the mean.

| Parameter      | Control knees | Arthritic knees |
|----------------|---------------|-----------------|
|                | Baseline      | Post            | Baseline | Post |
| BF (mL/min/100g) | 7 ± 3         | 7 ± 2           | 11 ± 6   | 15 ± 6* |
| HbO2 (µM)      | 68 ± 20       | 63 ± 15         | 69 ± 19  | 60 ± 23 |
| Hb (µM)        | 34 ± 9        | 34 ± 9          | 38 ± 12  | 47 ± 16 |
| StO2 (%)       | 66 ± 2        | 65 ± 4          | 66 ± 2   | 55 ± 11 |

*Statistically higher (p < 0.05) than baseline blood flow.

The blood flow measured at baseline and after induction of arthritis on the control and inflamed knees are depicted in Fig. 4. The larger 3rd quartiles in Fig. 4(b) were due to higher baseline blood flow in the right knee of one animal. Since there was no sign of injury or swelling in the knee, the higher flow likely reflects natural variability in perfusion between subjects, which further illustrates the value of using a quantitative technique, rather than relying on relative approaches [52]. The ability to quantify joint perfusion would be valuable when conducting longitudinal studies in which different groups are compared (e.g., for evaluation of treatment strategies) or a single subject is followed over time (e.g., in treatment monitoring).
Fig. 5. Longitudinal trends in flood flow measured on the control (healthy) and inflamed knee of one animal. Day 0 and 3 were baseline measurements and inflammation was induced right after the measurements on day 3.

Figure 5 shows an example of the longitudinal trends in the data in the control and arthritic knees. For this example, the mean blood flow measured on the control knee was 4.1 ml/min/100g and the standard deviation across all measurements were 2.1 ml/min/100g. Importantly, this variability was less than the typical changes induced by arthritis and it was possible to clearly distinguish between inflamed and control joints as shown in Fig. 4.

4. Discussion

The purpose of the present study was to investigate the sensitivity of joint blood flow to arthritis and compare it to that of Hb, HbO2, and StO2—the typical physiological parameters measured by DOS. Experiments were conducted on a rabbit model of inflammatory mono-arthritis using a non-invasive multi-wavelength time-resolved DOS technique. The study revealed that arthritis causes a decrease in joint oxygen saturation and HbO2 content. In contrast, blood flow and Hb increased in inflamed joints; however, only blood flow reached statistical significance.

These results are in agreement with the known pathophysiology of inflammatory arthritis. Chronic inflammation of the synovial membrane that lines the joint—the main feature of RA—is known to promote cell infiltration, which results in hypertrophy of the synovial lining. Normally 1-3 cell layers, the thickness of the synovial membrane increases to more than a dozen cell layers in RA [21]. The augmented synovial cell mass induces an increased oxygen demand that exceeds supply, resulting in hypoxia [21,22,24]. Another important characteristic of RA is angiogenesis—a direct consequence of the hypoxia, which is a potent signal for neovascularization [24]. The formation of new blood vessels enables a supply of nutrients and oxygen to the augmented inflammatory cell mass and thus, contributes to the progression and perpetuation of the disease. However, despite the increased vascularization, the RA joint remains hypoxic since the neo-vessels lack the functionality that permits autoregulation of blood flow in response to tissue demand. Importantly, it has been shown (using invasive probes) that the partial pressure of oxygen is lower in inflamed than healthy joints [23]. Furthermore, it is well documented that chronic hypoxia leads to increased tissue BF and lower oxygen saturation (StO2) [40,53]. Taken together, these findings and ours confirm the important role of hypoxia in inflammatory arthritis.

Furthermore, since the sample size of the study was small, type II error was a concern. To control for the small sample size, we conducted the following analysis. First, for each physiological parameter, the mean and standard deviation across all subjects was computed at baseline and after induction of arthritis for the control and arthritic knees. Thereafter,
G*Power statistical software was used to assess the effect size for each pair of data set (e.g., StO₂: Control knees at baseline vs Control knees at Post, Arthritis knees at baseline vs Arthritis knees after induction of inflammation) using their mean, standard deviation, and correlation computed with SPSS. We subsequently estimated the sample size that would provide a statistically significant effect at the level of \( p<0.05 \) with a power of 0.8. For conciseness, we only report results for pairs of data set for which a large effect size (effect size>0.8) was found. The analysis revealed that differences in blood flow between baseline and post had the largest effect size (1.79), and we estimated that a sample size of only 4 subjects is enough to detect a statistically significant difference \( (p<0.05) \) between perfusion in inflamed knees from baseline values with a power of 0.8. The physiological parameter with the second largest effect size was Hb (effect size of 0.92) and would require a sample size of 9 subjects to detect a statistically significant difference between control and inflamed joints with a power of 0.8. For comparison, 27 and 80 subjects would be required to detect a significant difference in StO₂ (effect size of 0.49) and HbO₂ (effect size of 0.28), respectively, between control and inflamed knees with the same power.

These findings are significant in a number of regards. This is the first report of a noninvasive optical method that can quantitatively measure joint blood flow. Quantification is important for longitudinal studies as it accounts for inter-subject variability in perfusion. Considering the current lack of adequate methods for RA treatment monitoring, this technique could play a significant role in the management of this debilitating disease. Note that there are other optical techniques—diffuse correlation spectroscopy (DCS) and changes in Hb and HbO₂ following venous occlusion—that can noninvasively measure deep tissue perfusion [54–57]. However, while it has been shown that the venous occlusion method can quantify blood flow in muscle [57], its ability to quantify joint perfusion has yet to be tested. DCS is typically used to measure perfusion changes, rather than absolute blood flow [54]. Although a recent report and our own work have demonstrated that DCS blood flow index is proportional to absolute blood flow [54,58], the use of DCS for monitoring joint blood flow has not been reported. It is also worth noting that the large proportion of bone in joint may significantly complicate the interpretation of DCS data acquired on joints because of the breakdown of the validity of the theoretical models used to interpret DCS measurements [59].

Although the results of this study clearly demonstrate that joint blood flow was the most sensitive to inflammatory arthritis, there are few limitations that could potentially prevent the generalization of these results. Among those limitations is the fact that Hb, HbO₂, and StO₂ were obtained from the absorption coefficients measured at the three laser wavelengths by assuming 70% water concentration in the knee joint. Water is the major constituent of tissue but its concentration in joint is not tightly regulated as it is in the brain. As such, the water concentration in the knee joint could vary, but would likely remain between 60 and 95% [60]. We evaluated the error that assuming a 70% water concentration would have on the above physiological parameters when its true concentration in the joint was 60 or 95%. The analysis indicated that this assumption would cause less than 4% error in any of the physiological parameters. Such a small error would likely not have a significant effect on the conclusions of the study. Furthermore, since the TR-DOS system had three wavelengths, it should theoretically enable to estimate the water concentration as well as Hb and HbO₂. However, we found that even with three wavelengths, it was difficult to extract a reliable water concentration, and assuming a known water concentration was necessary to achieve consistency in the estimation of Hb and HbO₂.

Another potential limitation of the study is the use of a theoretical model that assumes tissue homogeneity to analyze data acquired on the knee joint. We recognize that the knee joint is a complex structure comprised of soft tissue, ligaments, cartilage, bone, etc. However, the effects of tissue heterogeneity should be similar for all three absorption coefficients used to compute the physiological parameters. Thus, we expect the effects of tissue heterogeneity to be similar for all the parameters, and the conclusions of the present study should not be
affected by tissue heterogeneity. Future work will involve tomographic imaging to account for spatial variation in tissue composition. The challenge for tomographic blood flow imaging is sufficient speed to capture dynamic contrast enhancement in the tissue (Fig. 3). However, methods such as structured light illumination and single-pixel compressed imaging have the potential to meet this challenge [61].

The simulations showed that for a medium with the size and optical properties similar to those of the rabbit knee, assuming finite size in the directions of height and length, i.e. all the dimensions other than that of the two optical probes, has no significant effects on the accuracy of the recovered absorption coefficients. This is to say that tissue with a height of 40mm and length of 160mm can be considered optically infinite in those two directions. Thus, we only needed to account for the source-detector distance (i.e., the width of the knee). The width of the knee joint was measured with a clipper at the beginning and at the end of each experiment, and the average value was used as the source-detector distance in the data analysis. This approach enables to account for changes in the size of the knee joints during the arthritic period. As well, we compared tissue ICG curves obtained using the infinite-slab model and the modified Beer-Lambert law wherein the light attenuation was converted into changes in absorption coefficient using the optical pathlength derived from the TPSFs and the IRF. This analysis did not reveal any significant difference between the ICG concentrations obtained by the two approaches, which further confirms the validity of considering the knee joint as an optically infinite medium in the directions other than that of the source-detector. However, the authors acknowledge that the changing size of the knee joint can potentially cause inaccuracies and future improvements could include comparing the infinite slab model to simulations conducted on more realistic knee joints, obtained from MRI images of knees with a range of sizes.

5. Conclusion

A longitudinal study was conducted in a rabbit model of inflammatory arthritis. Joint blood flow, Hb, HbO2, and StO2 were quantified and their sensitivity to joint inflammation assessed. The results revealed that joint blood flow was more sensitive to arthritis than any of the other physiological parameters. Thus, joint blood flow should provide a more sensitive marker for monitoring rheumatoid arthritis than the typical physiological parameters obtained with DOS. Furthermore, the effect size analysis provides an objective means of estimating the sensitivity of the different physiological parameters to joint inflammation and could be used to guide the design of future studies.

Funding

This research was supported by an Internal Research Fund (IRF) from the Lawson Health Research Institute (14987).

Acknowledgments

The authors would like to acknowledge the technical support of Jennifer Hadway and Lynn Keenliside.