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REVIEW ARTICLE

Near Infrared Fluorescence Imaging for early detection, monitoring and improved intervention of diseases involving the joint

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Abstract

Joints consist of different tissues, such as bone, cartilage and synovium, which are at risk for multiple diseases. The current imaging modalities, such as magnetic resonance imaging, Doppler ultrasound, X-ray, computed tomography and arthroscopy, lack the ability to detect disease activity before the onset of anatomical and significant irreversible damage. Optical in vivo imaging has recently been introduced as a novel imaging tool to study the joint and has the potential to image all kinds of biological processes. This tool is already exploited in (pre)clinical studies of rheumatoid arthritis, osteoarthritis and cancer. The technique uses fluorescent dyes conjugated to targeting moieties that recognize biomarkers of the disease. This review will focus on these new imaging techniques and especially where Near Infrared (NIR) fluorescence imaging has been used to visualize diseases of the joint. NIR fluorescent imaging is a promising technique which will soon complement established radiological, ultrasound and MRI imaging in the clinical management of patients with respect to early disease detection, monitoring and improved intervention.

Keywords

Joint, near infrared fluorescence imaging, osteoarthritis, rheumatoid arthritis, sarcoma.

History

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Introduction

Optical imaging of the joints, using a class of near infrared (NIR) fluorescent dyes, is a novel imaging modality with the potential to be used in the clinic. It would complement very well with the established in vivo imaging technologies, magnetic resonance imaging (MRI), Doppler ultrasound, computed tomography (CT) and X-ray fluoroscopy, which are currently employed for diagnosing and monitoring diseases of the joint or include joint involvement. Inflammation plays a major role in nearly all diseases of the joint and joints suffering from arthritis are the leading cause of disability in adults (1). Rheumatoid arthritis (RA) is such a disease in humans, which is a consequence of chronic inflammatory processes (2). This eventually leads to the progressive and functional loss of mobility and long-term tissue damage in joints and extra-articular parts of the body. RA has one of the worst quality of life scores within chronic diseases (3). Early diagnosis is important to start treatments immediately, since many patients tend to develop joint damage within the first years of disease (4). The need for longitudinal monitoring is compounded by the fact that people often live with RA for 30 years or more. Furthermore, within 10 years of the start of the condition, at least half of the people with RA are unable to work fulltime (3). Osteoarthritis (OA) is another major pathology, where inflammation is associated with the aetiology of disease. OA can be caused by the wear and tear or mechanical trauma to the articular cartilage, the hydrated tissue that lines the ends of long bones in load bearing joints. Articular cartilage provides joints with a smooth, gliding surface, but it degrades with age and/or after certain events of infection or trauma.

Medical evaluation of the joint is currently based upon imaging of morphological and physiological features by established radiological, ultrasound, MRI and nuclear imaging. The recent introduction of optical imaging offers the prospect of monitoring molecular events. Important applications have been developed in this area where it can be used not only for diagnostic imaging or tracking activity in diseases, such as inflammation and joint degradation, but also for therapeutic purposes, such as therapeutic monitoring, stem cell migration and image-guided cancer surgery. Fluorescent optical imaging has the potential to become a complementary imaging tool where it is rapid, inexpensive, non-ionising and can be performed in real-time. This review will focus upon this new technique and the role that optical imaging can play to optimize detection and therapy. Also other disorders potentially benefit from optical imaging, such as systemic sclerosis and sarcoma. Using the latter case as an
example, we will discuss the interesting and promising field of NIR fluorescent image-guided cancer surgery.

The challenge of imaging the joint – early detection, disease progress monitoring and improved intervention

Current clinical imaging technologies that offer diagnostic information whereupon clinical decisions are made range from non-invasive modalities, such as X-ray, ultrasound, MRI and nuclear imaging, to more invasive techniques, such as arthroscopy. Radiological techniques are mainly used to investigate the pathophysiology of bones. For example, in RA it helps to visualize erosions and joint space narrowing, which are the late onset morphological changes in patients suffering from RA (5). Currently, using conventional imaging techniques, early diagnosis is still a difficult task (6). This is even more relevant for OA as inflammatory activity may be lower making diagnosing of disease more difficult in comparison with RA. Unfortunately, current clinical radiological imaging techniques can only detect OA when it is irreversible and the articular cartilage is already deteriorating (7). If OA can be detected early, especially in its reversible stage, it could provide physicians an important opportunity for intervention long before clinical symptoms are being manifested (8). MRI can complement radiological technologies in its ability to image soft tissues and to provide anatomical details by offering a high spatial resolution of the anatomy of the body (9). However, MRI can be associated with a low sensitivity where it is unable to relate the anatomy to disease activity (9).

High frequency ultrasound (US) improved the spatial resolution of techniques used in RA by allowing better detection of erosions than radiological techniques (10). Moreover, US can provide functional information on, for example, blood flow when using US Doppler. In the evaluation of synovial inflammation, vascular signals are useful for determining the degree of inflammation and thus the activity of disease can be monitored (11). Finally, US Doppler can also offer information on the onset on erosions (12).

Other imaging techniques that can be used to assess disease activity are nuclear imaging modalities such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT). Despite the use of $^{18}$F-FDG, management and early detection of RA have not been optimal due to the lack of specificity, since glucose uptake is not specific for diseases, but also occurs in other processes of the body with increased metabolism (9). Therefore, sensitive and specific methods of imaging are required for the early stage detection of RA. PET probes are synthesized without structural changes, but have to be synthesized on the spot since radionuclides like $^{18}$F-FDG are short lived. To do so, extensive and expensive infrastructure and logistics are needed including a cyclotron, hot-labs and radio-pharmacists. The short half-life of typical PET probes also limits its use, because the probes rapidly bind to their target and are quickly cleared from the circulation.

Clinically, if non-invasive imaging cannot determine a patient treatment strategy, more invasive imaging tools can provide extra information on the anatomy. Arthroscopy helps to visualize structures of the joint in real-time and provides further information about structures, namely, tendons, cartilage, bone, ligaments and muscles. Radiological devices or MRI can detect either indirect or directly administered contrast agents during arthroscopy to provide real-time moving images known as fluoroscopy. The contrast agent gadolinium can be detected by MRI and provides information on the synovial area, vascularity, permeability, and pre-existing joint effusion. Directly administrated gadolinium, where it is injected into the joint, makes imaging of small internal structures possible, with the disadvantage that only one compartment can be assessed. Indirect arthroscopy allows enhancement of all joint compartments, but also extra-articular enhancement is achieved as the contrast agent is injected intravenously (13). However, joint compartment distribution or joint fluid uptake can be slowed or limited by diseases, as is the case with synovial fibrosis (14). In other diseases it is known that diseased tissue becomes innervated due to penetration of vessels from the underlying bone, for example, diseased articular cartilage. Still the direct and indirect administered contrast fills up non-specifically, so the contrast signal can appear as a false positive signal (13). In short, arthroscopy allows observation of anatomy and its function, but no information is provided on disease activity and its location before structural changes occur.

Though current imaging modalities have proven their place among joint diagnostics, fluorescent optical imaging can complement these imaging tools by providing information on perturbations in specific molecular pathways (15). In the case of joint disease the key is to detect disease early and monitor disease activity to improve patient care and intervention. Therefore, optical imaging offers advantages over conventional imaging techniques because it is able to monitor disease activity in the short and long term, e.g. the progression of disease, and the visualization of specific targets to achieve optimal personalized patient care and treatment.

NIR fluorescent dyes

For fluorescent optical imaging (1) a fluorescent probe and (2) a camera system are needed. The technique exploits the use of light where a fluorescent dye is conjugated to a targeting moiety (together they form a probe) that can locate a process or a molecule of interest (16). Use of fluorescent dyes in the NIR region has multiple advantages compared to those that emit in the visible light spectrum.

Several advantages exist in using the NIR fluorescent technique over the conventional techniques: To gauge how optical imaging can complement the established in vivo imaging modalities, the potential and limits of NIR fluorescence have to be put into context. Making optical probes, compared to PET probes, is relatively cheap and easy since it just involves the conjugation of a fluorescent dye (fluorescein) to a specific moiety to make a specific probe. For optical imaging the conjugation of the fluorescent dye to the probe can alter the specific binding of the probe due to structural alterations making probe design a challenging task (17). However, targets and targeting moieties already validated by PET studies can be used in optical imaging. Furthermore, the tissue-scattering properties and absorption
of light must be taken into account (see Figure 1) since the optical properties of light with a wavelength between 400 and 650 nm are associated with reduced signal specificity for experimental probes. Also autofluorescence is relatively high in this part of the spectrum. Therefore, the preference for optical imaging is to use deeper penetrating, and more sensitive, NIR fluorophores, with an emission between 650 and 900 nm (18). The advantage of using NIR fluorescent dyes is that autofluorescence of tissue under investigation is low when using this wavelength range, which means that deeper-lying features (up to 10 mm) may be visualized. However, only a limited number of NIR fluorescent dyes are currently available for use in the clinic.

There are two different classifications of NIR fluorescent dyes for optical imaging; those that are non-targeted and those that are targeted (19).

**Non-targeted approaches**

The current FDA and EMA approved clinical dyes, indocyanine green (ICG) and methylene blue, are both non-targeting. In joint imaging, ICG plays an important role where it is employed to detect persistent synovitis in RA, which leads to massive joint destruction and eventually causes irreversible disability in patients. Early detection of synovitis means that immediate therapy can be given to improve short- and long-term outcomes (20–22). The uneven distribution of ICG in the joint visualized changes or a disturbed microcirculation (23). A fluorescence camera system, Xiralite® (Mivenion, Berlin, Germany) detects very low amounts of intravenously injected ICG and thus enables the visualization of very small disturbances of the microcirculation by mapping regionalized differences in signal intensities making RA in hands of five patients visible (Figure 2) (24). The use of ICG has also been extended to studying systemic sclerosis, a connective tissue disease affecting various organs including the peripheral vessels, which causes Raynaud’s phenomenon (25). The non-targeting action of ICG in these studies is due to inflammatory vascular leakage and the increased permeability for macroglobulins. Due to the interactions between ICG and serum proteins, ICG acts as a large molecule and causes perivascular accumulation when blood vessels are leaky.

**Targeted approaches**

For targeting, there are available NIR fluorescent dyes, such as Cy5.5, ZW800-1 and IRDye® 800CW, which are conjugated onto target molecules of interest. Two of those dyes, ZW800-1 and IRDye® 800CW, are currently in clinical trials for a variety of different medical applications. IRDye® 800CW (Li-Cor, Lincoln, NE) is manufactured under current good manufacturing practices (cGMP), has completed a toxicity study performed under good laboratory practice and has drug master files on record with US and European regulatory authorities (26). ZW800-1 is also available as a free dye and is produced under cGMP (27,28). It has also been conjugated onto cyclic RGD (cRGD), a well-known peptide that targets integrins in the neovasculature, which was used intra-operatively to identify colorectal tumors in subcutaneous and orthotopic animal models specifically and the ureter due to renal clearance (29). One of the earliest studies of NIR fluorescent in vivo imaging in the joints was the visualization of early experimental arthritis in a murine model of antigen-induced arthritis. The target was the F4/80 antigen present on the surface of macrophages infiltrating the inflamed synovial membrane. Imaging was performed using

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**Figure 1.** Before measurement of fluorescent signals, travelling light is subjected to reflection, scattering and absorption of the tissue under investigation.

**Figure 2.** Comparing quantitative fluorescence readout of three phases after injection (A, B and C) and a fluorescence ratio (between joints and the eponychium), synovitis could be identified and imaged. Reproduced from the publication of Schäfer et al. (24).
outcome. Alarmins have recently been shown to be locally

NIR fluorescent targeted approaches also include a

category referred to as active targeting using ‘‘smart’’

active molecule-based probes (ABPs). In these probes the NIR

fluorescent imaging, the presence and distribution

visible in vivo background of the fluorescent probe is lower than with

signal intensity between normal and OA joints

cathepsin B fluorescent ABP was used

present and an unquenched probe (32). Active targeting, therefore, offers

optical imaging shows better specificity and is less prone to

Monitoring degeneration of the joint

Degenerative joint diseases are prevalent particularly in the

elderly. OA is considered to be an effect of ageing where the

articular cartilage degrades. The initial stages of OA are

clinically silent and it is possible to be asymptomatic for

many years. However, when symptoms do occur and when
current radiological imaging techniques are capable of
detection, the deterioration of articular cartilage has already
occurred (7). If it were possible to monitor and assess the
disease states of OA, it would provide an important oppor-
tunity to intervene before the occurrence of significant
irreversible damage and clinical symptoms (8).

NIR fluorescent imaging linked to known biomarkers of
OA has shown promise in preclinical studies. The glycosa-
mimoglycans (GAGs) play an important role in the integrity of
articular cartilage. A study from Hu et al. showed that two
cationic NIR fluorescent dipicolylamine (DPA) probes, Cy5-
DPA-Zn and Cy7-DPA-Zn, could permeate perichondrium
and accumulate in cartilage matrix through binding to
abundant anionic GAGs (45). In vivo imaging revealed a
higher Cy5-DPA-Zn uptake and retention where GAG levels
are higher in young mice than in older mice.

Biochemical studies of OA have indicated that matrix

metalloproteinase-13 (MMP-13) promotes degradation of the
cartilage extracellular matrix (46,47). Its expression was also
found to be highly upregulated in degenerating cartilage (48).
The work of Lee et al. described a fluorescent technique using a
MMP-13 peptide substrate, a quenched NIR fluorescent
ABP, both in vitro and in rat models (49). This enabled the
visual detection of MMP-13 where the different stages of OA
can be readily monitored, imaged and analyzed by using a
dark-quenched fluorogenic probe.

Tissue engineering, nanotechnology and
regenerative medicine

Biomolecules measured in nanometres have opened the door
to many new healthcare applications giving rise to various
new branches of science encompassing nanomedicine and
nanotechnology. The underlying theme is that modification
of topography on a nanoarchitectural level can be used to
optimize biocompatible materials in their interaction with
cells. Tissue engineering, nanotechnology and stem cells
encompass a very broad topic where the underlying theme is
the use of nanotechnology and tissue engineering to create
functional interaction of artificial materials with biological
tissues or stem cells.
Orthopaedic tissue engineering has concentrated primarily on bone, whereas engineering of cartilage is gaining momentum rapidly including the seeding of mesenchymal stem cells (MSCs). Traditionally, the biochemical and structural characteristics of engineered tissues have been assessed through destructive lab techniques such as histology. However, the cell–biomaterial interactions that result in successful outcomes are not yet well understood and are difficult to observe. Optical visualization has been employed where its use has been successfully reported in the form of two photon microscopy (50), optical coherence tomography (51), and also whole body NIR fluorescent imaging (24). NIR fluorescent visualization in particular has now been employed at many different levels including labelling the biomaterial and/or for in vivo tracking of the cells. This allowed the better understanding of the detailed in vivo process of scaffold degradation, tissue formation and cell-based tracking can be better understood.

A recent study showed that functional biodegradable scaffolds covalently linked to a NIR fluorescent dye could be monitored where the degradation could be quantified in real-time (52). Optical imaging is also versatile enough to allow multimodality imaging where other imaging modalities can be employed simultaneously. The group of Kim et al. simultaneously observed tissue ingrowth by measuring vascularization using MRI in the same animal over a month. The monitoring of biological processes can also be extended to in vivo NIR fluorescent cell tracking which could offer further insight into cell-based therapies and the specific processes of cell function, migration, homing, differentiation and engraftment. MSCs are often used for their regenerative capacity within the joints. One study examined the in vivo migration of transplanted human MSCs into the marrow cavity of athymic nude rats with osteochondral defect (53). Their aim was to observe whether migrating MSCs could promote the healing of injured articular cartilage. Interestingly they used NIR fluorescent dye loaded nanoparticles, which another group had previously reported as being taken up by human MSCs without cytotoxicity (54). Fluorescence was maintained up to seven cell culture passages and the nanoparticles did not affect the expression of cell surface markers. The work from Lee et al. was the first report showing optical imaging of NIR fluorescent labelled MSCs inside the bone marrow cavity migrating to a defect in the joint in a time-dependent manner (55). The potential to utilize carriers to deliver therapeutic payloads has also been exploited and can be fluorescently-labelled in order to locate their position and monitor longitudinally. The work of Sandker et al. showed the presence of microsphere carriers of different sizes in articular joints (see Figure 3), where they have the potential to be used for local sustained drug delivery in vivo (56). The ability to visualize the microsphere enabled the group to show that these microspheres have promise as intra-articular drug delivery vehicles for the treatment of arthritic diseases.

**Sarcoma surgery: intra-operative fluorescent guidance**

Sarcoma, a group of cancers originating from mesenchymal cells, is still associated with poor survival and quality of life. The average long-term, disease-free survival of this category of cancer has remained relatively low over the last 20 years with 40–60% survival for all soft tissue sarcoma subtypes combined (57). Chondrosarcoma is one of the most common malignant cartilage-forming tumors whereas osteosarcoma is more prevalent as a primary bone tumor (58). Currently there are no biomarker tests available, so MRI and radiological imaging are often used to make a diagnosis. The treatment of sarcoma involves the consideration of neoadjuvant therapy, adjuvant therapy and surgical options. Sarcoma surgery is performed by surgeons, who rely mainly on their clinical judgment in combination with a radio diagnostic work up with CT and/or MRI. Resection of these malignancies is technically demanding as complete surgical resection can be difficult or sometimes even impossible, because of the close proximity of the tumor to vital structures and adjacent compartments. The goal of limb-sparing surgery for a soft tissue sarcoma of the extremity is to remove all malignant cells whilst preserving limb function. Any microscopic residual disease in the tumor bed will cause local recurrence. After surgery, the completeness of removal is evaluated by assessing the quality and thickness of this margin. During the last decades, the tumor margin after sarcoma resection has most often been classified according to Enneking’s classification, as intra-lesional, marginal, wide or radical/compartimental (59). The surgical goal is currently a margin with a thin rim of healthy tissue surrounding the tumor (60–62). However, the best margin width for sarcoma surgery is still unclear and there is no consensus as yet of what constitutes a safe margin.
A publication from Reijnders et al. detailed the use of two- and three-dimensional radiological image-guided surgery for soft tissue and bone sarcomas using computer-based image processing (63). The information was used to provide radiological data to the surgeon during the operation in a single comprehensive display while integrating intra-operative information into the same framework. Though the beneficial effect for patients was difficult to quantify as the hardware was used only on two patients, it showed more importantly that image-guided surgery could give a better perspective for resection of sarcomas.

More recently, the concept of using NIR fluorescent imaging agents to guide surgical resection of sarcomas in animal models has been reported. A series of NIR fluorescent cathepsin protease-ABPs were used to detect microscopic residual sarcoma in mice under a wide field-of-view optical imaging device. This was the first time that a primary sarcoma model system, also not transplanted, has been used for intra-operative imaging studies with a non-cleavable probe as a control (64). The optimal situation for resection of chondrosarcomas would therefore follow where a NIR fluorescent probe could be used in conjunction with a specific marker. A number of different MMPs have already been identified which are specific for chondrosarcomas, which could be implemented as ABPs for image-guided surgery (65,66). These results combined demonstrate the potential of image-guided surgery for intra-operative visualization to provide a preclinical platform for testing new imaging agents and systems.

**Conclusion and future clinical perspective**

Clinical imaging of the joint is currently based upon the monitoring of morphological and physiological features. The established in vivo imaging technologies of MRI, PET, Doppler ultrasound, CT and X-ray fluoroscopy, with some more frequently employed than others, have their various drawbacks. Most relate to issues of cost, sensitivity and specificity. The clinical management of patients with joint pathology requires early detection and better disease monitoring so that improved intervention can take place leading to successful patient outcomes. Radiological imaging has the disadvantage of only being able to detect late morphological changes, which may not appear until six months after disease onset.

The recent introduction of optical imaging now offers the opportunity to derive functional information, much earlier, on actual molecular pathway activity or to reveal concentration of molecular markers. The technology is rapid, inexpensive, non-ionising and is performed in real-time. The use of ICG, as described earlier, showed already the promise of the technique in patients with RA and OA. The clinical utilization of NIR fluorescent imaging is already starting to make inroads into the field of image-guided oncological surgery. The same intra-operative procedures could also be applied to resection of chondro- or osteosarcomas. However, even optical imaging has its own obstacles, which will need to be addressed prior to entering the clinic. The targeted probes, and especially ABPs, will also need to undergo complex and expensive clinical trials. In these trials relevant molecular biomarkers will need to be evaluated and show clinical relevance. Before clinical use also other aspects, such as toxicity and routes of administration, should be tested. Furthermore, advances in imaging hardware will be required to allow deeper interrogation into the tissue where currently optical imaging at high resolution and depth is limited.

Nevertheless optical imaging is a promising modality and there is no doubt that it will assist in the clinical management of patients with respect to early diagnosis and in response to new drug treatments. It is unlikely to displace any of the conventional imaging methods in the short-term future, but more likely it will become an additional technology that will complement established radiological, ultrasound and MRI imaging. Information involving anatomical details will remain the domain of the current established methods. However, where optical imaging will play a major role in joint care is in its molecular specificity and ability to diagnose early onset before symptoms materialize. We expect that measuring molecular activity, such as the degree and extent of joint inflammation, is a task that would be performed more optimally upon optical imaging platforms. Also cartilage degenerative markers and novel gene reporter designs made to monitor stem cell migration or differentiation will be sought after by clinicians in the future as we strive towards improving patient outcome by better visualization of the disease.

**Declaration of interest**

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