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SPOTLIGHT

Superparamagnetic iron oxide nanoparticles as a multimodal contrast agent for up to five imaging modalities

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The current gold standard method to find the sentinel lymph node (SLN) is lymphoscintigraphy, where ^{99m}Tc-labeled nanometer sized colloids are injected sub-dermally or peri-tumourally, and then imaged using a gamma camera/SPECT (before surgery) and a gamma probe (during surgery). A blue dye injected prior to surgery may serve to visualize the SLN intra-operatively. When the SLN is found, it is removed by invasive surgery and cancer infiltration is examined using histology. The lack of precise anatomic information in the scintigraphic images and the non-specificity of the tracer, however, often limit the pre-operative planning and the identification of the SLN.

Multimodal approaches in the clinic, such as the next generation hybrid systems based on combined MR/PET-systems, are expected to address several of these issues. In the pre-clinical setting, an even faster growing segment for medical imaging–multimodal systems including optical methods is concurrently emerging. Interestingly, there exist still no commercially available clinical contrast agent that can be used for even two modalities, and research has shown functionality for only a couple imaging modalities at a time. Our aim is to develop novel multimodality contrast agents and methodology for such systems to meet new needs for quantitative and high-resolution imaging. In this perspective, superparamagnetic iron oxide nanoparticles (SPIO-NPs) are emerging as a highly attractive approach.

SPIO-NPs can be observed by MRI as signal voids in the MR image and SPIO-NPs have been approved as MRI contrast agents for more than a decade with applications including lymph node detection. We propose the use of SPIO-NPs as a multimodal contrast agent by attaching radionuclides and fluorophores to the surface of the nanoparticles, for, respectively, SPECT, PET, and optical imaging methods. Moreover, the particles also inherently functions as contrast media for a new imaging technique called magnetomotive ultrasound imaging. Thus, the very same particles can be used as a contrast agent for up to five modalities, depending on the type of surface modification. We expect the following improvements for the work-flow of cancer patients undergoing lymph node surgery:

- One single injection of tracer, and thereby no timing issues experienced with the dye used today. This means less preparation time prior to surgery, and reduced risk of lymph edema due to excessive removal of colored lymph nodes.
- Improved staging due to truly combined information in a PET/MR system including tracer migration analysis.

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- A possibility to non-invasively determine metastatic spread in lymph nodes, in many cases eliminating the need for a surgical SLN procedure.

For sentinel lymph node surgery there are two major application examples, where a multimodal contrast agent is expected to be beneficial.

First, reliable staging of the cancer is important in order to choose the correct therapeutic method and the extent of the procedure. When the primary surgery is finished, one-third of all patients have had an axillary dissection with removal of the lymph nodes in the axilla due to spread through the lymphatic vessels to the SLN and other nodes in the vicinity. Patients who develop lymph edema usually are from this group. But there is also a more serious complementary risk that too few lymph nodes are removed in the case of metastatic infiltration. This is a typical example of the benefit of multimodal imaging: PET offers high sensitivity and accurate activity quantification and temporal information of the tracer, however lymph node anatomy cannot be revealed. Anatomical MRI, on the other hand, has excellent spatial resolution but is less quantitative, with considerable less contrast. By applying compartment modeling and time series imaging to the acquired PET data, additional information on the tracer migration from the injection site can be obtained. In combination with high-resolution MR new parametric images can be achieved that we anticipate can reduce the number of false-negative outcomes.

Second, for surgery guidance, optical techniques and ultrasound would be very helpful to the surgeon to find SLN in a less invasive way. Today, leakage of dye through the sentinel nodes makes it impossible for the surgeon to discriminate which nodes to remove. To perform the surgical procedure more accurately intra-operative optical and ultrasound imaging for SPIO-NPs can complement the initial staging. The key idea is then that the day after the PET/MR scan, the radioactivity of the tracer has decayed, but tumor specific NPs are still entrapped in the relevant lymph nodes. To limit the uptake mainly to the sentinel lymph node, the design of particle size and surface structure is crucial [1]. With prior attachment of fluorophores to the NPs optical imaging can be used. To extend the investigation depth magnetomotive ultrasound (MMUS) can be used—a technique that can be implemented in scanners used in the clinical setting today.

Ultrasound, being cost-effective and portable, is an excellent candidate as an intra-operative guide, but the SPIO-NPs particles are too small to be imaged directly with conventional ultrasound equipment. A solution is to utilize the magnetic properties of SPIO-NPs particles to obtain image contrast. By virtue of a time-varying magnetic field, induced particle movement is used to differentiate particle-laden tissue, as particles also move their immediate surroundings. This was first shown for optical coherence tomography, and later using a clinical ultrasound scanner. The drawback with the approach of conventional movement detection schemes such as Doppler processing used for flow measurements is a strong sensitivity to movement artifacts. Members of our team, however, recently developed a MMUS algorithm based on quadrature detection and phase gating at the precise frequency of nanoparticle displacement, hereby effectively suppressing unwanted movements at other frequencies [2].

This opens up for an entirely new modality to be used together with those others already suited for SPIO-NPs. With improved preoperative staging and guidance during surgery, operations would be less invasive with removal of less lymph nodes, warranting improved quality of life. More importantly, with combined PET and MR imaging, or using for instance tissue elastic parameters derived from magnetomotive ultrasound imaging, information on metastatic spread in the lymphatic system may be obtained. Which of either magnetomotive ultrasound or optical imaging is the most suitable technique for clinical use remains to be determined.

Madru et al. and Evertsson et al. have shown that it is possible to detect SPIO-NPs laden SLNs with MRI, SPECT, and optical techniques in the same animal [3, 4], as well as with MRI and MMUS, also in the same animal [2]. The preclinical model studied was clinically relevant showing drainage through the lymphatic system, as well as showing accumulation of SPIO-NPs in the sentinel lymph node. Contrast agent was injected subcutaneously on the dorsal side of the right hind paw of Wistar rats, after which the animals were free to move about in their cages. After 24 h the SPIO-NPs were accumulated primarily in the popliteal node, located at the posterior surface of the right knee, and imaging was performed (Fig. 1).

We envisage that by combining the best property of each imaging modality an improved identification and localization of the SLN will be achieved and thereby there is potential for improved diagnosis for spread of cancer in, e.g. breast cancer and malignant melanoma. The combination of high anatomical resolution MRI with high sensitivity and quantitative PET/SPECT, will increase the possibility to ensure a more accurate localization of the SLN before surgery. During surgery more portable techniques such as optical imaging and magnetomotive ultrasound can be used as a guide, visualizing the very same contrast particles. The pre-requisite is a contrast agent that is truly multi-modal where the same contrast particle can be detected by relevant imaging modalities. Surface modified SPIO-NPs satisfy this for SPECT, PET, MRI, optical

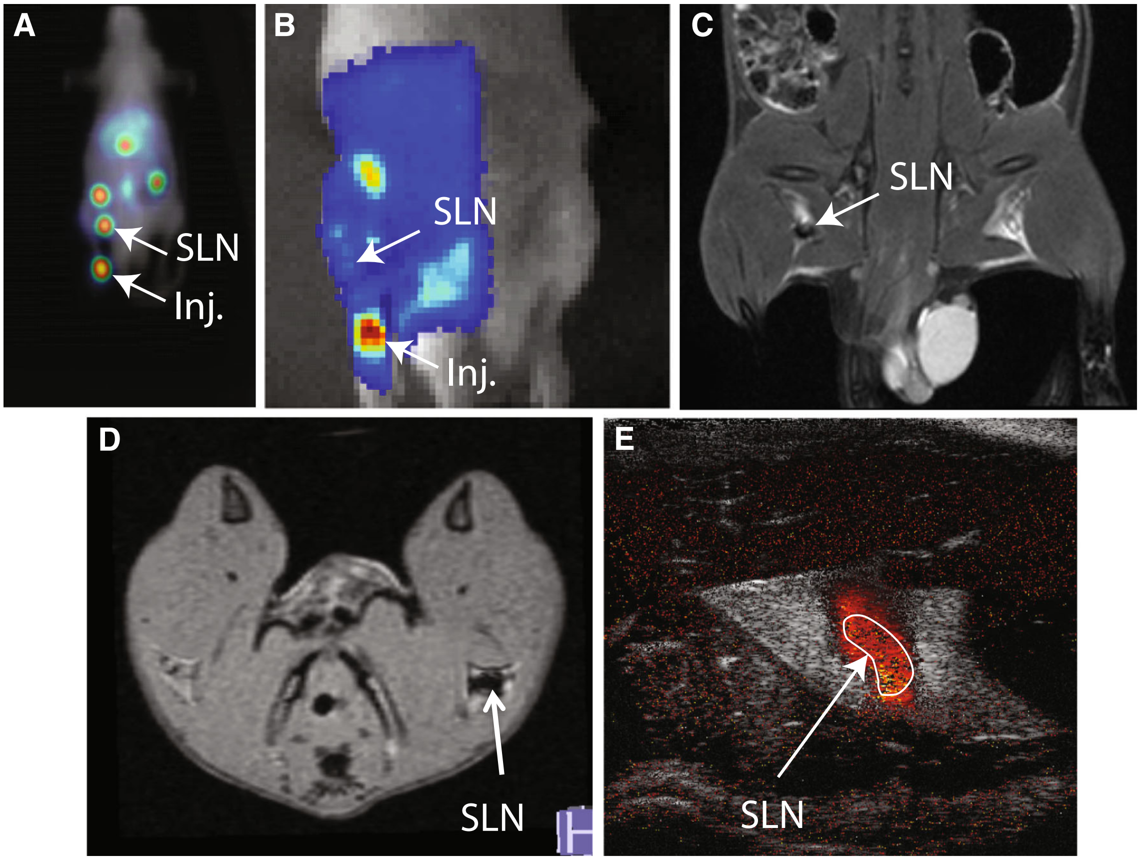

Fig. 1 Multimodal imaging using SPIO-NP as a contrast agent or contrast agent vehicle. **a** SPECT imaging, with the sentinel lymph node and injection site marked with *arrows* denoted "SLN" and "Inj", respectively. **b** Fluorescence imaging with sentinel lymph node and injection site marked as in **a**. **c** MRI with a signal void in the sentinel lymph node containing SPIO-NP (*arrow*). Compare the contralateral side where no SPIO-NPs are present. **d** MR-image for comparison with magnetomotive ultrasound imaging, **e**. The sentinel lymph node is marked with *arrows*, and also outlined with a white border in **e**. Colors in **e** code for magnetomotive displacement. **a**–**c** is from the same animal, while **d** and **e** are from another animal

techniques, and magnetomotive ultrasound, which we have shown in a pre-clinical model, however further validation is required before clinical use.

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Conflict of interest Tomas Jansson, Stefan Andersson-Engels, Freddy Stahlberg and Sven-Erik Strand declare no conflict of interest. Sarah Fredriksson is board member of Genovis AB and Geccodots AB.

Human and Animal Studies This article contain studies with animal subjects. All animal experiments were conducted in compliance with the national legislation on laboratory animals' protection and with the approval of the Ethics Committee for Animal Research (Lund, Sweden).

References

1. Kjellman P, in't Zandt R, Fredriksson S, Strand SE (2014) Optimizing retention of multimodal imaging nanostructures in sentinel lymph nodes by nanoscale size tailoring. Nanomedicine 10:1089–1095
2. Evertsson M, Kjellman P, Cinthio M, Fredriksson S, in't Zandt R, Persson HW, Jansson T (2014) Multimodal detection of iron oxide nanoparticles in rat lymph nodes using magnetomotive ultrasound imaging and magnetic resonance imaging. IEEE Trans Ultrason Ferroelectr Freq Control 61:1276–1283
3. Madru R, Kjellman P, Olsson F, Wingårdh K, Ingvar C, Ståhlberg F, Olsrud J, Lätt J, Fredriksson S, Knutsson L, Strand SE (2012) 99 mTc-labeled superparamagnetic iron oxide nanoparticles for multimodality SPECT/MRI of sentinel lymph nodes. J Nucl Med 5:459–463
4. Madru R, Tran TA, Axelsson J, Ingvar C, Bibic A, Ståhlberg F, Knutsson L, Strand SE (2013) ^{68}Ga-labeled superparamagnetic iron oxide nanoparticles (SPIONs) for multi-modality PET/MR/Cherenkov luminescence imaging of sentinel lymph nodes. Am J Nucl Med Mol Imaging 15:60–69