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Moriaki Kusakabe, Michael Douek

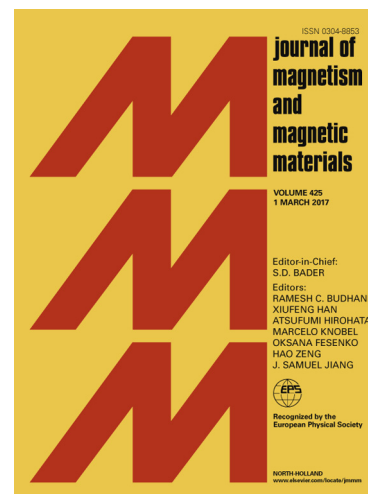
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Feasibility study evaluating a magnetic marker in an *ex-vivo* porcine model.

Mirjam C L Peek, MSc ^{1,a,b}, Ali Zada, MSc ^{1,a,b,c}, Muneer Ahmed, PhD MRCS ^{a,b}, Rose Baker, PhD ^d, Masaki Sekino, PhD ^e, Moriaki Kusakabe DVM, PhD, ^f Michael Douek, MD FRCS ^{a,b}.

¹ Joint first authors

^a Research Oncology, Division of Cancer Studies, King's College London, Guy's Hospital, London, Great Britain

^b King's College London, Research Oncology, Division of Cancer Studies, Guy's Hospital, London, Great Britain

^c Institute for Biomedical Technology and Technical Medicine, Universiteit Twente, Enschede, The Netherlands

^d School of Business, 612, Maxwell Building, University of Salford, Salford M5 4WT, Great Britain

^e Department of Electrical Engineering and Information Systems, Graduate School of Engineering, University of Tokyo, Tokyo, Japan

^f Advanced Technology Research Laboratory, Research Centre for Food Safety, Graduate School of Agriculture and Life Sciences, University of Tokyo, Tokyo, Japan

Email: mirjam.1.peek@kcl.ac.uk, private.alizada@gmail.com, muneer.ahmed@kcl.ac.uk, rose.baker@cantab.net, sekino@bee.t.u-tokyo.ac.jp, kusabmrl@gmail.com, Michael.douek@kcl.ac.uk.

Corresponding author for pre-publishing queries:

Mirjam Peek, mirjam.1.peek@kcl.ac.uk, +44 (0)20 7188 0743

Corresponding author for manuscript queries and reprints:

Michael Douek, Professor of Surgical Oncology, michael.douek@kcl.ac.uk, +44 (0)20 7188 6380

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ABSTRACT

Aims: The magnetic technique using a magnetic tracer and handheld magnetometer was successfully evaluated in breast surgery. Residual tracer at the injection site can cause susceptibility artefacts on breast magnetic resonance imaging (MRI), therefore for lesion localization a solid marker would be preferable. Four magnetic markers were developed for localization to evaluate its clinical applicability.

Methods: Comparison was made of the maximum magnetic counts and artefact-volume on MRI.

Results: All markers were successfully detected, the spring marker showed the highest mean magnetic counts (627.8 ± 400.2 , mean \pm SD) with unequal variances ($p < 0.001$) and the butterfly marker showed the smallest mean artefact-volume ($11.1 \pm 2.3 \text{ cm}^3$) with a significant difference between markers ($p = 0.049$).

Conclusion: Localization using a magnetic marker is feasible and further evaluation is required within a clinical trial.

HIGHLIGHTS

- Magnetic markers developed for lesion localization were evaluated in a porcine model
- Magnetic count and visibility on imaging modalities (MRI, US, etc.) were evaluated
- The spring marker showed the highest mean magnetic counts (627.8 ± 400.2 , mean \pm SD)
- The butterfly marker showed the smallest mean artefact-volume ($11.1 \pm 2.3 \text{ cm}^3$)
- The magnetic markers were visible on ultrasound and mammography

KEY WORDS

Magnetic technique, magnetic markers, lesion localization, magnetometer, porcine model.

ABBREVIATIONS

BCS Breast conserving surgery

MRI Magnetic resonance imaging

SLNB Sentinel lymph node biopsy

US Ultrasound

WGL Wire guided localization

BACKGROUND

In early stage breast cancer patients, breast conserving surgery (BCS) has been adopted as the standard treatment, replacing mastectomy for the majority of patients.(1, 2) Due to the wider use of breast cancer screening and advanced imaging modalities, breast cancer is diagnosed at an earlier stage. This has led to an increase in screen detected non-palpable tumours, resulting in an increase in patients eligible for BCS. In the United Kingdom, approximately 33% of all breast cancer cases are non-palpable. (3, 4) In addition, more patients are offered primary medical treatment further increasing the number of impalpable tumours and suitability for BCS. (5) The current standard technique for treatment of non-palpable lesions is wire guided localized (WGL) excision. Drawbacks of WGL, include wire migration and unacceptable high re-excision rates which can exceed 20%. ⁴ These drawbacks have encouraged the development of novel techniques for lesion localization.

One of these novel techniques is the magnetic technique which was first evaluated for sentinel lymph node biopsy (SLNB). The magnetic technique utilizes a handheld magnetometer to localize sentinel lymph nodes following a subcutaneous injection of super paramagnetic iron-oxide based magnetic tracer. Its non-inferiority to the standard for SLNB was first demonstrated in the SentiMAG Multicentre Trial (6) and these findings have now been confirmed by several other studies. (7-13) The first in women use of the magnetic technique for non-palpable lesion localization was performed in the MagSNOLL trial subsequent to successful implementation of the technique in porcine models. (4, 14) This trial showed a 100% success rate in detecting the non-palpable lesions with a re-excision rate of only 10%, suggesting that the magnetic guidance may improve the quality of BCS by improving intra-operative guidance.

A drawback of the magnetic technique for SLNB are susceptibility artefacts at the injection site seen on subsequent breast magnetic resonance imaging (MRI). (15) In a minority of patients who require breast MRI follow-up, this may negatively impact on the clinical value of breast MRI in view of data loss. However, the magnetic tracer is not visible on conventional imaging modalities (mammography and ultrasound (US)), or on histology. For lesion localisation a solid magnetic marker rather than a magnetic tracer would be preferable, since a solid marker can be excised entirely and artefacts on subsequent MRI will be avoided. Four different magnetic markers were developed, to be injected into non-palpable lesions under US guidance and readily detectable with a handheld magnetometer. Since in certain clinical circumstances (e.g.: prior to primary medical therapy) magnetic markers are left *in-vivo* for a period of time and breast MRI is used to monitor response to treatment, we also evaluated artefacts resulting from these different solid magnetic markers. We evaluated the potential clinical applicability of

these magnetic markers within an *in-vivo* porcine model comparing detectability of the magnetic markers using a magnetometer, artefact volume on 1.5T MRI and visibility of the markers on other imaging modalities.

METHODS

The magnetic markers were evaluated in an *in-vivo* porcine model, which replicated the human size, vasculature and lymphatic drainage. This study was conducted at the IRCAD institute (Strasbourg, France) and at King's College London (London, United Kingdom). Ethics approval was granted for animal experimentation by the IRCAD Ethics Review Board (Strasbourg, France; ref: 38.2011.01.008). All procedures performed during this study were in accordance with institutional guidelines.

Magnetic markers

Four ferromagnetic markers, made from stainless steel 430, each with a length of 4.5 mm and width of 0.9 mm were developed and evaluated. The magnetic markers had the following shapes: (1) solid, (2) barrel, (3) spring and (4) butterfly and an amount of iron equivalent to 0.2, 0.05, 0.02 and 0.01 ml of magnetic tracer, respectively (*Table 1*).

Magnetometer

The handheld magnetometer used for the detection of the markers was developed by the University of Tokyo (Tokyo, Japan) and consists of a Hall effect sensor inside a permanent magnet. (16) The magnetic flux density measured by the magnetometer is displayed with a scale ranging from 0 to 999 magnetic counts; where a higher count is displayed when the magnetometer is in close proximity of a material with a strong magnetic field. An earlier prototype of this magnetometer was previously validated in a trial evaluating the magnetic technique of SLNB against blue dye as a standard. (9)

The procedure

Four mini-pigs were anesthetized and used to conduct the different *in-vivo* experiments. The magnetic markers were inserted into the target area using an 18 gauge spinal needle, consisting of an inner and outer needle. The inner needle was removed slightly and the magnetic marker was placed at the tip of the outer needle and sealed off with bone wax. Once the outer needle was injected into the target tissue, the magnetic markers were injected by pushing the inner needle back into the outer needle. Four

of the same magnetic markers were injected at a standardised distance subcutaneously into each of the mini-pigs, close to the nipples, with convenient distance between each injected magnetic marker (*Figure 1*).

Subsequently *in-vivo* measurements were performed and repeated three times with the handheld magnetometer. The tissues containing the magnetic markers were excised keeping a wide margin of tissue surrounding each magnetic marker. All tissues were marked and frozen overnight, in preparation for MRI assessment in the morning.

All tissue materials were imaged using a 1.5T MRI scanner (Siemens MAGNETOM Aera) and a body coil. T1 TSE and T2* sequences were used with SE 2.5/3.25 mm; RT 300 ms; TE 11 (T1) and 6.15 (T2); 256x256 pixels, FA 140 (T1) and 18 (T2); NA 1 (T1) and 2 (T2); echo train 3 (T1) and 1 (T2) (*Figure 2*). The markers were subsequently located with the handheld magnetometer and removed, apart from four – one of each subtype – which were fixed in formalin and preserved for further examination at King's College London. After excision of the remaining magnetic markers, four pieces of tissue were scanned with MRI as a control to determine if there was any tissue artefact after removal of the magnetic markers. To evaluate the extent of the artefacts, a 3D model of the tissue and of the artefact was constructed using the MRI images (*Figure 3*). The volumes of the 3D artefacts were measured and compared. Segmentation, volume determination and evaluation of the artefacts was performed using MATLAB R2015a (MathWorks, United States), and Blender software (V2.76, Blender Foundation, The Netherlands). In addition, the preserved tissues were imaged whilst in their container using X-ray. The excised tissues were too small for US evaluation. Therefore, US assessment was undertaken by scanning fillets of chicken into which the magnetic markers were injected.

Statistics

The counts obtained for the different magnetic markers and the artefact volumes were statistically evaluated using analyses of variance after homogeneity between results was ruled out using Levene's test. For equal variances a one-way ANOVA with Tukey HSD post-hoc test was performed and for unequal variances a Welch F-test with Games-Howall Post-hoc test. All statistical analyses were performed with IBM SPSS statistics 22 (SPSS Inc., Chicago, IL, USA).

RESULTS

Magnetic marker detection

A total of 16 magnetic markers were injected in four mini-pigs. All magnetic markers were successfully identified and descriptive statistics of the obtained magnetometer counts can be found in *Table 2A*. The mean highest magnetic counts were found with the spring (627.8 ± 400.2) and the lowest with the butterfly marker (261.7 ± 167.9).

Levene's test showed that the groups have unequal variances ($p < 0.001$). The Welch F-test showed that the magnetometer counts are significantly different between groups ($F(3, 23.2) = 4.2, p = 0.02$). Using the post-hoc Games-Howell test, only one pair with significantly different mean counts was found; the butterfly and spring marker ($p = 0.05$) with the spring having the higher magnetometer counts. All other pairs showed no significant difference between their mean counts (butterfly-barrel ($p = 0.08$), solid-butterfly ($p = 0.24$), spring-barrel ($p = 0.46$), solid-spring ($p = 0.90$) and solid-barrel ($p = 0.94$)).

MRI artefact

All artefacts caused by the magnetic markers were visible on T1 TSE and T2* scans and were heart shaped (*Figure 2 and 3*). Descriptive statistics of the MRI artefact volume measurements per marker can be found in *Table 2B*. The mean largest artefact volume was seen with the barrel ($22.5 \pm 9.1 \text{ cm}^3$) and the smallest with the butterfly marker ($11.1 \pm 2.3 \text{ cm}^3$). After excision of the magnetic markers, no magnetic artefacts were found on MRI with any of the magnetic markers.

Levene's test showed that the groups had equal variances ($p = 0.307$). A one-way ANOVA showed that the volumes were different between groups ($F(3, 12) = 3.5, p = 0.049$). However, Tukey's HSD post hoc test showed no significantly different pairs (butterfly-barrel ($p = 0.06$), butterfly-spring ($p = 0.08$), solid-butterfly ($p = 0.16$), solid-barrel ($p = 0.93$), solid-spring ($p = 0.96$) and spring-barrel ($p = 0.99$)).

Other imaging modalities

All magnetic markers were visible on X-ray and US (*Figure 4*). On X-ray the magnetic markers can be seen as an area of increased whiteness and on US the magnetic marker is visible as an area with increased echogenicity and a lack of echogenicity behind the magnetic marker.

DISCUSSION

There are several commercially available markers for lesion localization but most markers are made from titanium, platinum or non-magnetic stainless steel and detected by either US or mammography but not by magnetometers, hence the need to develop magnetic markers. (17) One study evaluated the magnetic MaMaLoc marker in combination with a handheld magnetometer in 15 patients and reported an identification rate of 100%. (18) No re-excision rates were reported for this study. The magnetic markers developed and evaluated in this study were easily detectable with the handheld magnetometer, with high counts. The highest magnetometer counts were found with the spring markers and the lowest with the butterfly markers. A significant difference ($p=0.05$) was found between these two magnetic markers in terms of magnetometer counts. Furthermore, the spring, barrel and solid markers were easier to inject than the butterfly marker due to their barrel-like shape. With regards to the MRI artefact, the largest artefact volumes were observed with the barrel markers and the lowest with the butterfly markers. Statistically, there was no significant difference between the butterfly markers and the other magnetic markers. Migration of the markers could not be assessed, as movement of the markers could not be determined against a tumor center or other certain location. However, during excision of the markers, it was difficult to remove the marker within the tissue and precise excision guided by the magnetometer was required. Our team uses a disposable spinal needle and bone wax to inject the markers; this is a cheap and easy way of applying the technique compared to using inducers. The butterfly marker has the smallest artefact on MRI but low magnetometer counts which could render deep lesions challenging to detect. From a clinical perspective, the optimal magnetic marker is the solid marker since it has both a smaller artefact on MRI and has a higher magnetometer count.

The feasibility of the magnetic technique was successful for lesion localization in patients with non-palpable breast cancer within the MagSNOLL trial although this was using a different magnetometer (Sentimag, Endomagnetics, UK). Recent discovery of the post-operative MRI artefacts due to magnetic dye residue makes localization with a solid magnetic marker a more clinically attractive procedure. Post-operative MRI is not an issue when the magnetic markers are used for pre-operative localization of impalpable tumors, as the magnetic marker will be excised during surgery and no artefact will remain. The magnetic markers were all visible both on X-ray imaging and under US, which ensures confirmation of correct placement and excision of the magnetic markers respectively under X-ray and US guidance. However, intra-operative US scanners might not have the same spatial resolution as those available at the radiology department and the magnetic marker might be more difficult to detect. In addition, as patients require subsequent SLNB, an additional tracer injection is necessary on the day of surgery. If

the MRI artefact due to residual magnetic tracer still poses a problem, SLNB can be performed using other tracers. (19-21)

Patients requiring pre-operative MRI imaging, for instance patients undergoing primary chemotherapy, are eligible for localization using these magnetic markers but since the artefacts on MRI are large and will impact on the ability to report on response to treatment, the magnetic markers should be placed immediately prior to surgery, after completion of primary chemotherapy. It might also be possible to decrease the artefact, by making the magnetic markers thinner or by lowering the iron content of these magnetic markers. Furthermore, by using specific MRI sequences the MRI artefact might be sufficiently suppressed. (22, 23)

For BCS, complete excision of the tumor is required and when this is not achieved, patients are offered additional surgery for re-excision. The MagSNOLL trial achieved a re-excision rate of just 10% suggesting that by improving localization using the magnetic technique, the precision of the BCS technique could also be improved. The magnetic counts provide the surgeon with a better sense of direction than with wire-guidance and the counts are proportional to the distance from the magnetic marker. Intra-operative US has already been shown in a randomized controlled trial to reduce re-excision rates and this could be further improved for impalpable lesions by combining US with the magnetic markers.

Other promising alternatives to WGL include radio-guided surgery – including radio-guided occult lesion localization, radioactive seed localization, and sentinel-node and occult-lesion localization. These have been shown to be effective for occult lesion localization, with high localization rates (in the range of 89.4 to 100%) and excellent surgical outcomes including a decrease in operation times, decrease in re-operation rates, and lower margin involvement.(24) However, implementation as a standard has not yet been established as the oncological benefit has not been satisfactorily confirmed.(4, 25) Furthermore, use of radioactive therapies requires specialized logistics such as a nuclear medicine department and the centre needs to be in close proximity to the nuclear reactor due to short half-lives of the used nanocolloids. For radio-guided occult lesion localization and sentinel-node and occult-lesion localization the required maximum amount of time between operation and injection of tracers is 24 hours, which complicate the logistics in planning the surgery. This technique therefore still remains at specialized centres. (4, 24)

Compared to radio-guided surgery and WGL, magnetic markers are not bound by limited time between injection and operation. The flexibility in operation planning logistics, the lengthy shelf life and ease of

use of the magnetic markers evaluated in this study highlight the need to evaluate these magnetic markers within a clinical trial in breast cancer patients. However, first further experiments are required to determine if these markers are safe to use by evaluating marker migration and leaching to surrounding tissues. Once markers are proven to be safe to use, a feasibility trial can be setup.

CONCLUSION

The magnetic technique using a magnetic marker and a magnetometer is feasible within *in-vivo* porcine experiments. The visibility under US and X-ray shows promise in the clinical use of the magnetic markers, however preoperative MRI imaging poses a challenge as artefacts due to the magnetic markers are too large for routine use prior to primary chemotherapy. Further clinical evaluation is needed preferably in a non-inferiority trial comparing against radio-guided occult lesion localization, and or WGL.

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Table 1. Characteristics of ferromagnetic markers used during experiments.

Table 2. Descriptive statistics of (A) magnetometer counts for each magnetic marker and (B) the magnetic resonance imaging (MRI) artefact volume measurements per marker.

Figure 1. Injecting markers and excision of tissue: (A) injection of the magnetic markers into the tissue spinal needle delivery method, (B) identification of peak magnetometer counts at site of marker, (C) excision of tissue containing magnetic markers and (D) excised tissue prepared for magnetic resonance imaging.

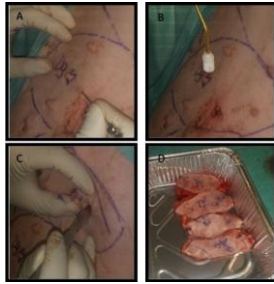


Figure 2. Magnetic resonance imaging (MRI) of the markers in excised porcine tissue: (A) T1 MRI sequence, (B) T2 MRI sequence after excision of the markers (from left to right: solid – spring – butterfly – barrel), (C) T1 MRI sequence showing an artefact (arrows) at the location of the butterfly markers and (D) T2 MRI sequence showing an artefact (A) at the location of the butterfly markers.

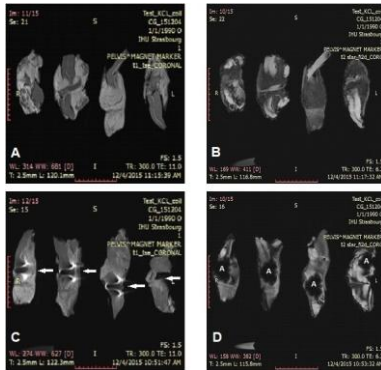
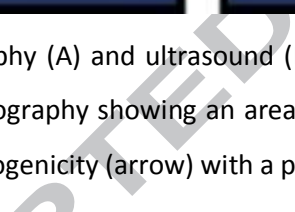


Figure 3. T2 magnetic resonance imaging (MRI) was used to segment a 3D image of the porcine tissue and artefacts caused by the butterfly markers with (A) T2 MRI image showing the artefact (A), (B) 3D segmented tissue (green) and artefact (blue), (C) segmented tissue and (D) segmented artefact.



phology (A) and ultrasound (B) of the same area. The histology (A) shows an area of necrosis (arrow) with a positive immunohistochemical reaction for the presence of the virus.

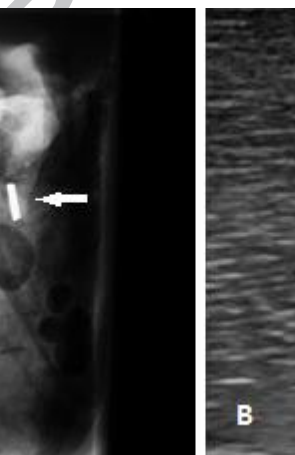


Table 1. Characteristics of ferromagnetic markers used during experiments.

Name	Solid	Barrel	Spring	Butterfly
				
Length	4.5mm	4.5mm	4.5mm	4.5mm
Diameter	0.9mm	0.9mm	0.9mm	0.9mm

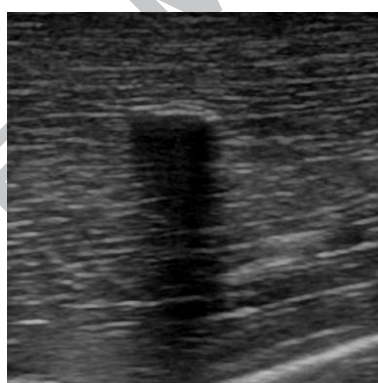
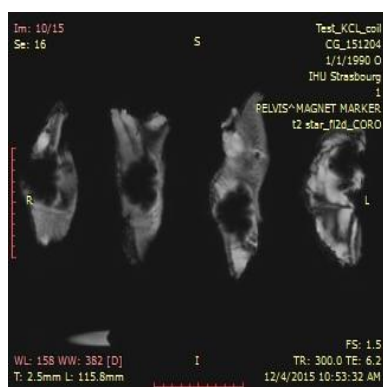
Table 2. Descriptive statistics of (A) magnetometer counts for each magnetic marker and (B) the MRI artefact volume measurements per marker.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower	Upper		
A. Descriptive statistics of the obtained magnetometer counts per marker								
A Solid	12	514.3	411.6	118.9	252.7	775.8	35.0	999.0
B Barrel	12	439.8	173.8	50.2	329.4	550.2	290.0	740.0
C Butterfly	12	261.7	167.9	48.5	155.0	368.4	10.00	480.0
D Spring	12	627.8	400.2	115.5	373.5	882.1	100.0	999.0
Total	48	460.9	330.0	47.6	365.1	556.7	10.00	999.0
B. Descriptive statistics of the MRI artefact volume measurements per marker								
A Solid	4	20.1	5.0	2.5	12.1	28.2	12.9	24.4
B Barrel	4	22.5	9.1	4.5	8.1	37.0	11.6	33.7
C Butterfly	4	11.1	2.3	1.1	7.5	14.8	9.3	14.5
D Spring	4	22.0	3.8	1.9	16.0	27.9	17.8	27.0
Total	16	18.9	6.9	1.7	15.3	22.6	9.3	33.7

HIGHLIGHTS

- Magnetic markers developed for lesion localization were evaluated in a porcine model
- Magnetic count and visibility on imaging modalities (MRI, US, etc.) were evaluated
- The spring marker showed the highest mean magnetic counts (627.8 ± 400.2 , mean \pm SD)
- The butterfly marker showed the smallest mean artefact-volume (11.1 ± 2.3 cm³)
- The magnetic markers were visible on ultrasound and mammography

GRAPHICAL ABSTRACT



Four ferromagnetic markers were developed for lesion localisation and evaluated the potential clinical applicability of these markers within an *in-vivo* porcine model comparing (a) maximum magnetic counts, detected with a handheld magnetometer; (b) artefact volume on 1.5T MRI; and (c+d) visibility of the markers on other imaging modalities. This study showed that magnetic localisation using a ferromagnetic marker is feasible within an *in-vivo* porcine model and further evaluation is now required within a clinical trial.

75 words

ACCEPTED MANUSCRIPT