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Doppler Ultrasound-Visible SignalMark Microspheres are Better Identified than HydroMARK® Clips in a Simulated Intraoperative Setting in Breast and Lung Tissue

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Abstract

Background.—Preoperative breast and lung markers have significant drawbacks, including migration, patient discomfort, and scheduling difficulties. SignalMark is a novel localizer device with a unique signal on Doppler ultrasound.

Objective.—We aimed to evaluate intraoperative identification of SignalMark microspheres compared with HydroMARK® clips. We also assessed the safety and efficacy of SignalMark in the lung.

Methods.—Twelve breasts of lactating pigs were injected with SignalMark or HydroMARK® by a breast radiologist, and subsequently identified using a standard ultrasound machine by three surgeons blinded to marker location. Time to identification of each marker was recorded, with a maximum allotted time of 300 s. To further demonstrate efficacy in lung parenchyma, a second cohort of pigs underwent lung injections.

Results.—A total of eight SignalMark markers and four HydroMARK® clips were placed in pig breasts. Overall, the surgeons correctly identified SignalMark 95.8% of the time (n = 23/24) and HydroMARK® clips 41.7% of the time (n = 5/12) within 300 s ($p < 0.001$). The mean time to identification was significantly faster for SignalMark, at 80.8 ± 20.1 s, than for HydroMARK®, at 209.4 ± 35.2 s ($p < 0.002$). For the lung injections, all 10 SignalMark markers were visible on Doppler ultrasound at the time of placement, and at the 7- and 21-day time points.

Conclusions.—Surgeons identified SignalMark in significantly less time than HydroMARK® clips in a simulated intraoperative setting, and SignalMark was easily viewed in the lung. These results suggest that SignalMark is a feasible option for efficient intraoperative localization of non-palpable breast and lung tumors using ultrasound guidance.

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INTRODUCTION

More than one-third of all breast tumors are non-palpable at diagnosis.[1] At the time of image-guided biopsy, a marker clip is deployed within the lesion to guide future localization. The majority of non-palpable breast tumors are localized with wires, radio frequency reflectors, or radioactive implanted seeds.[1-4] For wire localization, the entrance site in the skin of the breast is not always directly over the lesion; therefore, surgical dissection may be more extensive or less cosmetically appropriate. Tumor localization without the use of a wire is appealing because of the risks of wire dislodgement, logistical issues of wire placement in the hours prior to surgical excision, and the discomfort and cost of an additional procedure for the patient.[5] Additionally, prior studies have found that ultrasound-guided resection of non-palpable breast tumors is superior to wire-localization techniques with respect to margin status.[6,7]

HydroMARK® clips were developed to minimize the need for an external wire marker as the gelatin around the clip expands to allow for easier visualization with standard B-mode ultrasound.[8] The clips can be inserted weeks in advance of the planned surgical excision and have demonstrated excellent intraoperative visibility. However, a study by Klein et al. noted problems with surgical excision in 51.6% (16/31) of procedures,[8] demonstrating the need for further improvements prior to wide implementation of this technique.

SignalMark is a novel marker based on hollow microparticles that have a unique signal on Doppler ultrasound.[9] Our group has previously shown in rabbit experiments that microsphere-injected tumors were excised with significantly less marker migration versus traditional wire localization.[9] To date, we have not compared localization of SignalMark to the commercially available HydroMARK® clips. In this study, we evaluated the feasibility of intraoperative identification of SignalMark Doppler ultrasound-visible microspheres compared with the B-mode ultrasound-visible HydroMARK® clip in a simulated intraoperative setting.

Lung Nodule Localization

With recent evidence-based indications for lung cancer screening with low-dose computed tomography from the National Lung Screening Trial,[10] there is great need for minimally invasive, low-cost, convenient methods of lung nodule localization. Current preoperative localization techniques for lung lesions include wires, coils, and dyes.[11] As with breast mass localization, wire localization entails the usual difficulties of post-placement migration, discomfort for the patient, and logistical difficulties in scheduling placement immediately prior to surgical resection. Additional problems include pneumothorax and hemorrhage during wire placement,[12] as well as difficulty in intubating a patient who often cannot lie supine due to wire placement through the posterior thorax. An ultrasound-visible marker placed well ahead of surgery could alleviate many of the above issues with wire localization. However, it is traditionally difficult to ultrasound the lung due to the air within the parenchyma and airways; therefore, in a second experiment, we sought to test the SignalMark microspheres in lung parenchyma to confirm the feasibility and ease of use in pulmonary tissue.

METHODS

SignalMark Microsphere Construction

The preparation of similar microspheres is described in earlier publications.[13-15] The SignalMark microspheres used in this study consisted of a 2 mg/mL concentration of 2 μm ultrasound-visible spheres dispersed in a gelatin pellet. Three 5 mm pellets, one of which was wrapped with a radiographic coil, were inserted in a standard 14-gauge injector. The gross appearance and size of the breast and lung markers are show in electronic supplementary Fig. 1.

Breast Procedure

Two lactating female Yorkshire 200 kg pigs were used in the study and chosen because the breast size and tissue were felt to be most similar to human breast tissue compared with other readily available animal models. Lactating pigs were selected because they have more breast tissue than non-lactating pigs, in order to simulate the depth of a human breast. The pigs were anesthetized using general endotracheal anesthesia with isoflurane inhalational anesthetic, and were subsequently placed in the lateral decubitus position, exposing the breast tissue on one side. A total of 12 breasts were injected with eight SignalMark pellets and four commercially available HydroMARK® clips. More SignalMark markers were injected than HydroMARK® clips because several different formulations were under evaluation (one marker pad (gelatin/2 μm spheres) 1.6 mmD × 15 mmL, 1 marker pad (gelatin/2 μm spheres) 1.6 mmD × 6 mmL, 1 marker pad (gelatin/2 μm spheres) 1.6 mmD × 6 mmL with 1.2 mmD wire coiled 1.6 mmD × 2mmL). Both the HydroMARK® and SignalMark were allowed to hydrate for 45 min. All markers were placed by a board-certified, fellowship-trained breast imager using a standard 16-guage injector device (shown in electronic supplementary Fig. 2). The surgeons were blinded to placement of the breast markers. After placement, each of the three surgeons (one breast surgeon and two general surgery residents) individually searched for each marker using a standard ultrasound machine. One half of the ultrasound screen was set to B-mode and the other half was set to Doppler ultrasound mode during marker localization. After the study was complete, the pigs were sacrificed according to standard protocols. The study was performed in accordance with Institutional Animal Care and Use Committee (IACUC) practices and controls at California Medical Innovations Institute, San Diego, CA, USA.

The percentage of markers located and the time to identification of each marker was recorded, with a maximum allotted time of 300 s for marker identification. The mean time ± standard error to identification of each marker was calculated. The two-sample *t*-test and Fisher's exact test were used to analyze the data, and all data were analyzed using SPSS version 24.0 (IBM Corporation, Armonk, NY, USA).

Lung Procedure

Four Yorkshire 60 kg pigs were utilized for the lung injections. The animals were intubated and anesthetized with isoflurane general anesthetic and monitored with continuous pulse oximetry and telemetry for distress. The lung injections were performed using a 19-gauge introducer with a 20-gauge needle. The injections were performed under fluoroscopic guidance to confirm placement in the lung after a sham 20-gauge core biopsy. Ultrasound imaging was performed 10 min after injection and at the time of sacrifice. Two animals were sacrificed at 7 days and the remaining two at 21 days post-procedure. At the planned time of sacrifice, the animals were anesthetized, and B-mode and color Doppler ultrasound imaging were performed through the thoracic wall to observe the implant sites. The animals were then euthanized. The skin, ribs, and pleura were removed to expose the lung surface, and the area of marker placement was imaged with Doppler ultrasound from the lung surface. Sections of lung that contained SignalMark were then excised and reviewed microscopically by a pathologist to assess for histopathologic effects.

RESULTS

Breast Procedure

The appearance of each marker under ultrasound visualization is shown in Fig. 1. Under standard B-mode ultrasound, each marker looks similar (Figs. 1a and b); however, under Doppler mode, the SignalMark microspheres generate a robust, highly colored signal (Fig. 1c). Overall, the surgeons correctly identified SignalMark 95.8% of the time ($n = 23/24$) and HydroMARK® clips 41.7% of the time ($n = 5/12$) within 300 s ($p < 0.001$). Figure 2 shows the time to localization of each breast marker by each of the three surgeons. Assuming a maximum allotted time of 300 s, the mean time to identification was significantly faster for SignalMark, at 80.8 ± 20.1 s, than for HydroMARK®, at 209.4 ± 35.2 s ($p < 0.002$).

Lung Procedure

Overall, the animals showed no adverse reaction from the SignalMark implants. Respiratory monitoring of the animals during the injections revealed no signs of respiratory distress. A total of 10 injections were performed in four lungs of four pigs, with no pneumothorax noted. There were no postoperative mortalities. Two animals were sacrificed at 7 days post-injection, at which time all markers (5/5) were visible on Doppler ultrasound and on gross inspection of the lung surface. Figure 3 shows representative Doppler ultrasound images from the 7-day time point. The remaining two pigs were sacrificed at 21 days post-procedure per protocol, and, again, all markers (5/5) were visible on Doppler ultrasound and on gross inspection of the lung surface. Figure 4 shows representative Doppler ultrasound images from the 21-day time point. SignalMark appeared on the lung surface as a blue-gray mark that was distinct from the surrounding lung tissue (Fig. 5a). Histopathology of the implant site showed an expected inflammatory response. The typical foreign body response consisted of macrophage infiltration of the biopsy site, and encapsulation for digestion and transfer for elimination of the marker materials (Figs. 5b and c).

DISCUSSION

Overall, SignalMark microspheres were more rapidly and accurately identified than HydroMARK® clips in the breasts of lactating pigs. On average, SignalMark was found in less than half the time than HydroMARK®, and this difference was statistically significant. In the lung experiment, SignalMark markers were identified 100% of the time on Doppler ultrasound immediately after insertion, as well as at the 7- and 21-day time points.

Furthermore, SignalMark left a grossly visible blue-gray mark on the lung surface to confirm placement location, and no adverse events were noted.

Current widely implemented tumor localization techniques such as wire placement are fraught with difficulties, such as wire migration or transection, patient discomfort, suboptimal incision location, and logistical issues with coordinating two procedures on the same day, typically in different physical locations (breast imaging in an outpatient location and surgery in the hospital or surgery center).[5,16] Benefits of wire localization include the low cost and wide availability and familiarity to radiologists and surgeons. Radioactive seed localization (RSL) was created to avoid several of the pitfalls of wire localization. The RSL technique permits placement of the seed up to 5 days prior to planned surgical excision, and early studies reported decreased positive margins versus wire localization.[3] A more recent randomized controlled trial by Lovrics et al. refuted the improvements in positive margin status, but did report faster operative times, less patient discomfort, and greater ease of use by surgeons for RSL.[4] However, overall, the evidence is insufficient to date to support RSL as superior to wire localization.[1]

A newer method on the market, using non-radioactive, microimpulse radar technology and a reflector called SAVI SCOUT®, was introduced in recent years as a potential replacement for wire localization and RSL.[17-19] The system allows placement of the reflectors any time before surgery and is US FDA-approved as a permanent implant. Intraoperatively, a handpiece is used to locate the reflector and guide excision. After removal of the specimen, a radiograph is taken to confirm removal of the reflector and marker clip placed at the time of biopsy. The published prospective trial by Cox et al. showed favorable patient, radiologist, and surgeon experience, with a 16.8% re-excision rate for positive margins.[18] This method avoids the use of wires and radioactive material; however, the technology still has several limitations, including cost, interference from halogen operating room lights, deactivation with electrocautery, and difficulty in locating tumors >4 cm deep or within a hematoma.[18]

Alternative localization methods using intraoperative ultrasound (IOUS) avoid the need for wires, radioactive material, and specialized hand-held detectors. HydroMARK® clips are one such FDA-approved device in clinical use. Intraoperative visualization is reported to be excellent, but extrusion of the marker from the gelatin was a frequent complication during surgical excision in one study, and migration of the marker was also noted.[8] A more recent 2016 study by Gentile et al. in 107 patients found 100% success with excision of the maker, and statistically equivalent positive margins and specimen size compared with wire localization.[20] The authors concluded that HydroMARK® was a feasible and safe alternative to wire localization techniques.

SignalMark microspheres were designed to improve IOUS identification while still avoiding the problems inherent with wire localization and RSL. They are visible under standard B-mode ultrasound as prior markers, but also create a colorful signal under Doppler ultrasound, allowing for more rapid identification with any standard ultrasound machine. SignalMark is relatively inexpensive and can be placed at the time of initial biopsy to avoid a second procedure. SignalMark is currently under FDA review, but is designed for permanent implantation, with resorption occurring after 12 weeks, and animal studies to date have shown the microspheres to be visible for 12 weeks after implantation. Other markers are limited by tumor depth, but SignalMark is visible at any depth that can be imaged with ultrasound. Additionally, surgical dissection can be redirected as needed intraoperatively with repeated ultrasound imaging as excision proceeds, which is difficult with RSL because the exact depth of the tumor is not precisely known with this modality.

As in breast surgery, lung lesion localization continues to evolve. Wires are well-known to be associated with migration, pneumothorax, hemothorax, conversion to thoracotomy, patient discomfort, and day-of-surgery logistical issues. [12,21-23] IOUS has emerged as the preferential method of intraoperative localization due to its low cost, avoidance of irradiation, and minimally invasive technique.[11] IOUS has been noted to be safe and effective for lung nodule localization; however, its use has been limited as it is technically difficult to learn. A few studies have found it to be more accurate than palpation alone,[24-26] and in one study it reduced the rate of conversion to full thoracotomy.[24] Therefore, if IOUS was easier to learn and utilize, it could potentially replace other methods for lung nodule localization.

In our study, the SignalMark microspheres were readily visible in the lung on Doppler ultrasound immediately after placement and up to 3 weeks later. Therefore, it is feasible that SignalMark could be placed during an initial pulmonary biopsy or at any point several weeks prior to planned surgical excision, facilitating scheduling on the day of surgery. IOUS could then be used during thoracoscopic surgery or mini-thoracotomy to verify lesion location prior to resection. SignalMark also leaves a grossly visible mark when placed near the lung surface, which could be used for secondary verification intraoperatively. Testing is needed in humans, but this initial animal study found no adverse events, such as pneumothorax or significant hemorrhage.

Limitations of the present study include the relatively small sample sizes. In addition, both HydroMARK® and SignalMark were only hydrated for 45 min due to concerns over prolonged anesthesia for the animals, which may affect the ultrasound imaging, but it should affect both markers in a similar manner as they are both hydrogel-based. However, we demonstrate the feasibility of the SignalMark device in breast and lung applications. Strengths are the animal model used is as similar as possible to human tissue size and consistency, as well as the surgeons being blinded to the location of marker placement. In practice, surgeons have the benefit of preoperative mammographic and ultrasound imaging to guide localization. Further studies will be needed to validate the feasibility of SignalMark in humans, and excision can be performed safely with acceptable rates of positive margins and other complications.

CONCLUSIONS

In a simulated intraoperative setting using a swine model, surgeons located SignalMark microspheres in significantly less time than the HydroMARK® clips. SignalMark was also readily visible in lung tissue at the time of insertion and weeks later. These results suggest that SignalMark is a feasible option for efficient intraoperative localization of non-palpable breast tumors and lung lesions using ultrasound guidance. SignalMark has the potential to be more convenient for surgeons and patients, require less operative time, and avoids the complications associated with current localization methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SYNOPSIS

SignalMark microspheres were located in significantly less time than HydroMARK® clips in the breasts of lactating pigs. SignalMark was also easily viewed in swine lungs at insertion, and at 7 and 21 days post-insertion.

FIG. 1.

Images of (**a**) HydroMARK® and (**b**) SignalMark obtained with B-mode ultrasound, (**c**) SignalMark's appearance using Doppler ultrasound

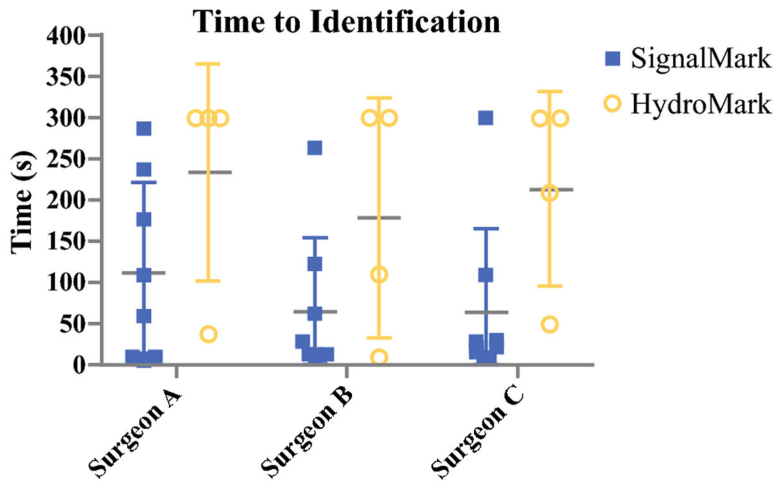

FIG. 2.

Time to identification of each implanted marker by three blinded surgeons. The center line of each whisker plot indicates the mean time to localization.

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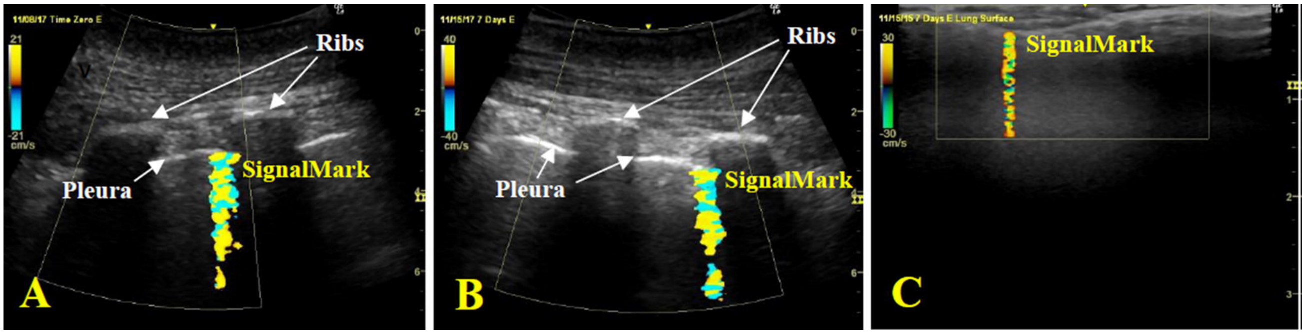

FIG. 3.

Representative Doppler ultrasound images of SignalMark at (**a**) time of insertion, (**b**) from the skin surface 7 days after insertion, and (**c**) from the lung surface 7 days after insertion

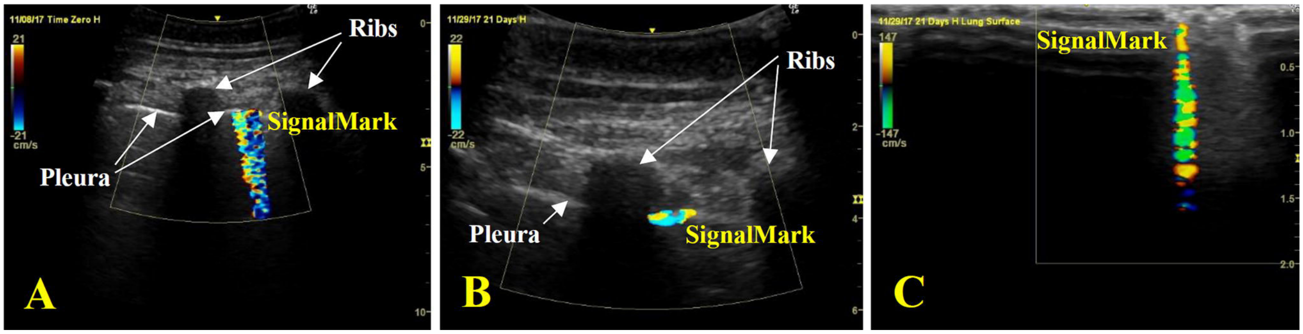

FIG. 4.

Representative Doppler ultrasound images of SignalMark at (**a**) time of insertion, (**b**) from the skin surface 21 days after insertion, and (**c**) from the lung surface 21 days after insertion.

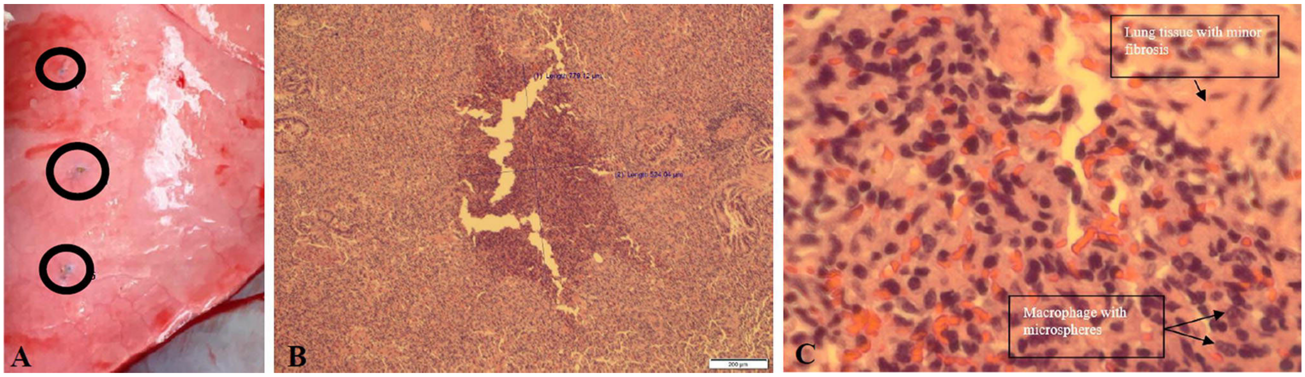

FIG. 5.
(**a**) SignalMark injection sites in one of the swine lungs at 21 days post-injection, (**b**) Histopathologic sections of the SignalMark implant site after 3 weeks showed chronic infiltrates at low power. A small amount of fibrosis was present on the fringe of the implant site, and near complete degradation and assimilation of the biomaterial (hydrogel) was noted. (**c**) Macrophage encapsulation of the microspheres was visible at high power.