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Utilization of iron (III)-doped nanoshells for in vivo marking of nonpalpable tumors using a VX2 rabbit model

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Abstract

BACKGROUND: We aimed to evaluate the potential for ultrasound (US) visible biodegradable nanoshells (NS) as an alternative to wire-guided localization for nonpalpable tumors in vivo.

METHODS: VX2 tumor was injected in bilateral thighs of 22 New Zealand rabbits and after 5 to 10 days, 1 tumor was marked with a wire as a control and the contralateral tumor was injected with 1 mL of 500 nm gas-filled silica NS under Doppler US. Tumors were excised after 24 hours. Chi-square was used for significance, $P = .05$.

RESULTS: One rabbit was excluded on postoperative day 1 due to equipment failure, no ill effects were observed from the NS. The NS were used to localize and resect 100% of marked tissue, 4/21 wires were displaced ($P < .05$).

CONCLUSIONS: We have shown that preoperatively injected US visible silica NS can be successfully used to mark nonpalpable tumors in vivo more consistently than WL.

Breast conserving surgery is the standard treatment for early-stage breast cancer and to 25%–30% of the diagnosed and treated breast cancers are nonpalpable requiring preoperative localization for successful resection.[1–4] Current strategies for marking these tumors include using a wire or implanted radioactive seeds to guide the surgeon to the biopsy-proven tumor.

The current gold standard and most frequently used method for localization is image-guided wire localization (WL). This technique relies on a thin hooked wire that is preoperatively inserted into the tumor under ultrasound or stereotactic guidance by a radiologist to guide the surgeon to the lesion. WL has inherent limits to precise localization

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because the external portion is vulnerable to displacement, and it only provides a single dimension of reference for a 3D tumor volume; consequently, reported rates of positive margins range from 14% to 47%.[5] To minimize the risk of displacement, the wire must be placed shortly before the procedure. Patients also report high levels of discomfort even associated with the wire[6]. Other techniques that address some of these concerns include radio-guided seed localization (RSL) with an internally placed seed. Unfortunately, RSL has been shown to have similar high rates of positive margins requiring re-excision and or mastectomy[5,7,8] Just as with "wire only", with "RSL only" a single plane of the tumor can be marked with the seed consistent with the continued high rates of positive margins[9] The irregular growth of nonpalpable tumors is postulated to contribute to the high rates of positive margins for both techniques.[9,10]

Intraoperative ultrasound-guided lumpectomy has demonstrated excision rates comparable to WL.[11] Studies reveal that up to 95% to 100% of the nonpalpable lesions can be successfully located with ultrasound.[12] This present study demonstrates the utility of localizing nonpalpable tumors with ultrasound visible gas-filled nanoshells (NS) to allow for rapid intraoperative localization and excision of nonpalpable tumors. This technique allows for the tumors to be marked multiple days prior and does not rely on radioactive seeds or vulnerable external wires.[13,14]

Methods

Nanoshells

The 500-nm hollow ultrathin iron (III)–doped silica NS were synthesized as previously described.[15–17] The hollow silica NS are subjected to at least 3 cycles of vacuum desorption followed by perfluoropentane filling. The perfluoropentane gas filled NS are suspended in degassed water at a concentration of 4 mg/mL.

Tumor model

The VX2 rabbit model was employed as rabbits were deemed the smallest species available (a) with an area of tissue of sufficient size to be able to implant and excise a tumor without severely impacting the mobility of the animal, (b) have tumors which were nonpalpable but visible with ultrasound, and (c) have sufficient muscle mass to allow for negative margins to potentially be achieved. The VX2 rabbit model has the additional advantage that it can be introduced and grown in a consistent time line rather than forming spontaneously. This allows for multiple rabbits to have the same time line for injection, marking, excision, and evaluation for regrowth.

This study was approved by the Institutional Animal Care and Use Committee of the University of California San Diego. Twenty-two female New Zealand White rabbits age 10 to 12 weeks were housed in an approved animal housing facility and kept at 20°C with a 12-h light/dark cycle. The animals were fed a commercially pelleted diet (Harlan Tecklad) ad libitum. Rabbits were anesthetized with isoflurane gas with oxygen during the procedures and excisions of primary tumors.

After the prescribed 72-hour acclimation period, the rabbits were injected with 1 cc of VX2 slurry in bilateral rear thighs. The tumors were allowed to grow between 5 and 10 days to a nonpalpable tumor approximately 2 to 5 mm in size. Twenty-four hours before excision, each rabbit had 1 tumor marked using B mode ultrasound-guided wire placement to serve as the control. The contralateral thigh was marked with approximately .5 to 1.0 cc of 500-nm gas-filled silica NS with Doppler-guided ultrasound (Fig.1). During imaging of the NS, the US parameters were optimized to maximize signal from particles while reducing background in all cases, as is done clinically. A frequency of 7 MHz and a mechanical index of 1.9 were chosen for Doppler imaging parameters, as in previous studies.[13] The US machine employed was the Siemens Acuson Sequoia 512 with the 15L8 transducer which is traditionally used for breast US imaging.

The marking of the tumors in this model requires general anesthesia for the animals and recovery time was required before the second general anesthesia for tumor excision, thus the wires were implanted 24 hours before tumor excision. This approach allowed for both marking strategies to be evaluated in an equal fashion and although more time was allotted to pass between marking and excision than is clinically standard, the transfers and potential manipulation of the wire was limited to minimize the potential risk of wire displacement. In addition, this necessary strategy allowed evaluation of the NS as a potential tumor marker that could be decoupled with the day of surgery.

The tumors were excised under closely monitored isoflurane anesthesia. The wire side was excised first to minimize any manipulation of the wire. After the wire side was closed, the nanoparticle side was imaged with the optimized Doppler parameters. The tumor was excised based on the Doppler image. The excised tissue was imaged and evaluated for evidence of NS in the sample, as was the tumor cavity. All tissue with evidence of remaining NS was excised at that time (Fig. 2).

Post operatively, the rabbits were allowed to recover for approximately 3 weeks until the animals were sacrificed to allow for evaluation of regrowth in the separate tumor beds. All pathology specimens were evaluated by a board-certified pathologist and tissue from the surgeries were evaluated for evidence of excised tumor and positive margins, which was defined as tumor touching ink (Fig. 3).

Statistics

Chi-square statistics were employed to compare the success of excision between the wire and the NS as well as the percent regrowth at a P value of .05.