

Carbon coated superparamagnetic iron oxide nanoparticles for sentinel lymph nodes mapping

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ABSTRACT

Intra-operative lymphatic mapping and sentinel lymph-adenectomy (LM/SL) maps the lymphatic path from the primary tumor to the regional nodes and permits selective excision of the first sentinel lymph nodes. It is a well established technique to detect occult regional node metastases for melanoma patients and breast cancer patients. In continuing attempts to improve accuracy, most surgeons now combine a dye (such as carbon particles) and radiopharmaceuticals when performing LM/SL. We developed a proto-type of carbon coated superparamagnetic iron oxide nanoparticles (SPIO@C) for sentinel lymph nodes mapping. Compared with combining carbon particles and radiopharmaceuticals for performing LM/SL, there are a number of advantages with our approach: I. SPIO is an MRI contrast agent, thus pre-operative MRI may be used for LM/SL instead of gamma camera. There is no radiation associated with MRI, and MRI offers good tissue contrast and detailed cross-sectional images. II. There will be only needed one injection of SPIO@C nanoparticles, instead of administering two successive injections of radiopharmaceuticals and carbon particles. III. During the operation, an intra-operative MRI scanner can be used, or more conveniently by a simple magnetometer.

KEY WORDS

Regional lymph nodes; sentinel lymph node; carbon; MRI; superparamagnetic iron oxide

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The spread of some forms of cancer follows an orderly progression, spreading first to regional lymph nodes, then the next echelon of lymph nodes, and so on, since the flow of lymph is directional, i.e. some cancers spread in a predictable fashion from where the cancer started. In these cases, if the cancer spreads it will spread first to lymph nodes close to the tumor before it spreads to other parts of the body. Sentinel lymph node surgery is to see if the cancer has spread to the very first lymph

node (the "sentinel lymph node", SLN). If the SLN does not contain cancer, then there is a high likelihood that the cancer has not spread to any other area of the body. Lymph node dissection during surgery with microscopic evaluation is the most accurate method to determine lymph node metastasis.

Detection of occult regional node metastases commonly involves intra-operative lymphatic mapping and sentinel lymph-adenectomy (LM/SL). LM/SL technique maps the lymphatic path from the primary tumor to the regional nodes and permits selective excision of the first SLNs. Typically, carbon particles, or a blue dye such as isosulfan, is injected around the primary tumour and travels along the lymphatic vessels. The first lymph nodes to receive lymphatic drainage from the primary tumour are stained dark (carbon color) or blue (for isosulfan), and are harvested as SLN for histology assessment. Particles can pass through the lymphatic vessels but not the blood capillaries mainly due to the difference in permeability. Histology techniques of multilevel sectioning and immunohistochemistry enable the detection of small nodal tumour infiltrates in the SLNs. Directing the pathologist's attention to a region of the

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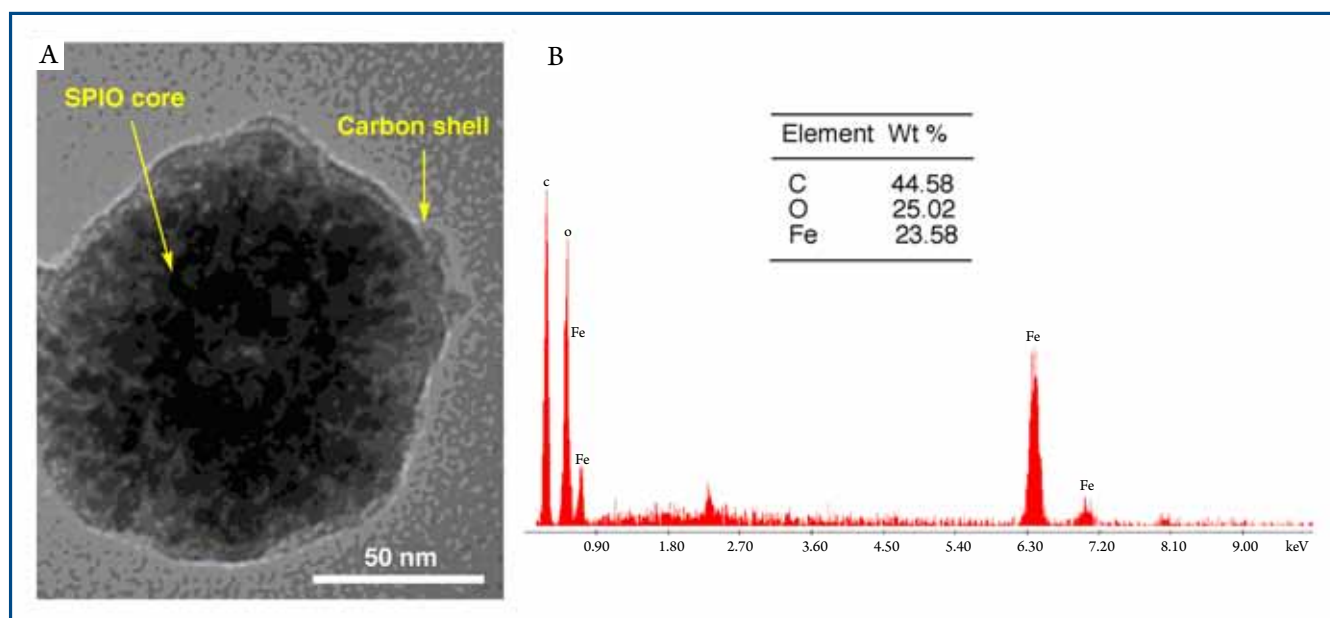


Figure 1. TEM image (A) and Energy-dispersive X-ray spectroscopy spectrum (B) of SPIO@C nanoparticle.

SLNs that is most likely to harbour metastatic deposits may increase the detection rate of small nodal tumour infiltrates (1). Patients with tumor-positive SLNs require complete lymph node dissection, while those without SLN metastases avoid the complications and costs associated with the complete lymph node dissection (2).

LM/SL is now well established for melanoma patients and for breast cancer patients (3-5). The technique is also being applied to a variety of other cancers, including colon cancer and non-small cell lung cancer (6,7). Isosulfan blue dye was a commonly used agent to identify the SLNs. However, the relatively rapid washout of dye from the SLN to successive nodes in the basin can lead to intraoperative misidentification of the sentinel node. Morton et al. introduced the use of carbon particles (carbon dye) for LM/SL (5). In medicine, carbon dye has served as both a diagnostic and a therapeutic agent for a long history. For example, carbon dye is used to mark polypectomy sites within the colon to facilitate endoscopic surveillance (8,9). Several studies have documented the safety of carbon dye in clinical use. Maruyama et al. reported no apparent adverse effects of intraperitoneal injection of carbon particle suspensions in more than 3700 patients (10). When used for lymphatic mapping, carbon particles are transported with the lymph from the site of injection to draining lymph nodes where they are phagocyted by macrophages in the subcapsular sinus, leading to a long-term, microscopically detectable mark on the lymph node. This is in contrast to the only temporary blue staining by isosulfan blue.

The site of carbon particles inside a tumour-positive SLN can be correlated with the location of nodal tumour infiltrates, therefore allowing “intranodal mapping”. SLNs are more likely to contain carbon dye as well as nodal tumour infiltrates (7). Isolated tumour cells are frequently located in the same nodal region as phagocytosed carbon particles (7). Hence, LM/SL using carbon particles supports the pathologist by marking the region inside a lymph node most likely to harbour tumour deposits further increases chances of detecting small nodal infiltrates (2,7).

In continuing attempts to improve accuracy, most surgeons now combine a dye (such as carbon particles) and radiopharmaceuticals when performing LM/SL (2). Preoperative lymphoscintigraphy is performed with a colloid agent and on the day of surgery. During surgery, the hand-held gamma probe directs the surgeon to the site of the radioactive (and dye -stained) SLNs (2). For lymphoscintigraphy, radiopharmaceutical injections are repeated every 20 min if required. Without gamma lymphoscintigraphy, carbon/blue stained afferent lymphatics and nodes can be difficult to find, because it is technically challenging, in a blood-stained surgical operative field, to identify lymph nodes and draining lymphatic vessels hidden in the adjacent fat. Radiopharmaceuticals alone can also be misleading in determining whether a node is sentinel (2). There is still no clear definition of a SLN when radionuclide tracer techniques are used, and the strong signal from radioisotope injected around the tumor, so-called “shine-through,” interferes with detection of radioactivity from lymph





Figure 2. SPIO@C *in-vivo* study in two rats. A: Rat 1, turbo spin echo T2-weighted image shows a SPIO@C containing lymph node (arrow) in right hind leg. B: Rat 1, gradient echo T2*-weighted image shows susceptibility effect (arrow) of the lymph node, confirming it containing SPIO. C: Dissection of rat 1 right leg shows a dark colored lymph node, corresponding to the lymph node in A&B. D: Rat 2, turbo spin echo T2-weighted image shows a SPIO@C containing lymph node (arrow).

nodes (11,12). Radiopharmaceuticals can cause radiation hazard not only to the patients, also to the health care staff. The logistics of injecting radiopharmaceuticals in the operating room present difficulties as well.

We developed a proto-type of carbon coated superparamagnetic iron oxide nanoparticles (SPIO@C) for SLN mapping. Mono-disperse SPIO nanoparticles was synthesized by a modified solvothermal method using $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ as precursor and ethylene glycol (EG)/ diethylene glycol (DEG) as solvent. The reaction was carried out in a sealed autoclave at high temperature. The particle size can be tuned (30-200 nm) by varying the EG/ DEG ratios and reaction time. To synthesize the SPIO@C core/shell nanoparticles, the SPIO (Fe_3O_4) nanoparticles were first

redispersed in glucose aqueous solution and then transferred to autoclaves and heated at high temperature. A carbonization process of the glucose was facilitated onto the surface of Fe_3O_4 nanoparticles under the hydrothermal conditions. The thickness (10-100 nm) of the carbon shell can be tuned by changing the reaction time. With our proto-type SPIO@C, the overall size is similar to those carbon particles currently in clinical use (100-150 nm) (Figure 1).

In a pilot study, SPIO@C suspension was injected to the right foot pad of two rats intradermally. MRI of the lower limb was performed 2 hour later, then the right lower limb was dissected and dark stained (carbon stained) lymph nodes were indentified. MRI demonstrated SPIO induced signal void for lymph nodes,



with gradient echo images demonstrating larger signal void area than the turbo spin echo images (Figure 2).

Compared with combining carbon particles and radiopharmaceuticals for performing LM/SL (2), there will be a number of advantages with our approach: I. SPIO is an MRI contrast agent (13,14), thus pre-operative MRI may be used for LM/SL instead of gamma camera. There is no radiation associated with MRI, and MRI offer good tissue contrast and detailed cross-sectional images. II. There will be only needed one injection of SPIO@C nanoparticles, instead of administering two successive injections of radiopharmaceuticals and carbon particles. III. During the operation, an intra-operative MRI scanner can be used, or more conveniently by a simple magnetometer (15). IV. The present used carbon nanoparticles cannot be dispersed well in water, instead, they can only be injected by mixing with a surfactant. Our proposed SPIO@C nanoparticles will have good water dispersibility.

Further optimization and validation studies are being carried out in our laboratories.

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