Dextran-coated \( \text{Fe}_3\text{O}_4 \) magnetic nanoparticles as a contrast agent in thermoacoustic tomography for hepatocellular carcinoma detection

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Abstract. Microwave-induced thermoacoustic tomography can provide a novel imaging modality for clinical detection. Significant progress has been made in the past several years in microwave-induced thermoacoustic tomography. In this paper, we investigate the feasibility of using dextran-coated \( \text{Fe}_3\text{O}_4 \) magnetic nanoparticles as a contrast agent in thermoacoustic tomography for hepatocellular carcinoma detection. Dextran-coated \( \text{Fe}_3\text{O}_4 \) magnetic nanoparticles administered intravenously are phagocytosed by resident Kupffer cells in normal reticuloendothelial system (RES) within the liver, but are not retained in tumor tissue. Consequently, there are significant differences in thermoacoustic signal intensity between normal RES and tumors, which result in increased lesion conspicuity and detectability. This provides the improvement of lesion-to-liver contrast for thermoacoustic tomography. A fast thermoacoustic computed tomography system with a multielement linear transducer array was used to image cancerous liver tissue with circular scanning. The results show that the system can provide molecular imaging with functionalized contrast agents for high-contrast detecting hepatocellular carcinoma and has the potential to become a novel approach for clinical diagnosis in the future.

1. Introduction

Pure microwave imaging has the advantage of good imaging contrast but suffers from poor spatial resolution due to the long wavelength of microwave[1-2]. Pure ultrasound imaging, an established medical imaging modality, can yield good spatial resolution but has poor contrast. Microwave-induced thermoacoustic computed tomography which is similar to photoacoustic imaging, combines the merits

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of both microwave imaging and ultrasound imaging, achieves excellent microwave absorption contrast and ultrasound spatial resolution, when compared to competing imaging techniques [3-8]. Various tissues present particular characteristics in their absorption spectra. Photoacoustic imaging can map optical absorption distribution whereas microwave-induced thermoacoustic imaging is related to the dielectric properties in the objects.

Thermoacoustic source wave is a result of microwave-induced thermal effect. A small temperature rise can be produced when a biological tissue is irradiated by a microwave pulse with adequate energy. Subsequently, the heated structure thermally expands and contracts, becoming a source of acoustic wave. By detecting the acoustic signals and via image reconstruction, thermoacoustic thermoacoustic computed tomography can be realized based on the heterogeneity in microwave absorption inside the object. This technique typically uses the endogenous contrast provided primarily by water content and ionic concentration in tissue [9]. While powerful, thermoacoustic tomography would benefit greatly from the development of exogenous contrast agents. Exogenous contrast agents would decouple contrast from penetration depth and open applications in which the endogenous contrast is insufficient, especially in molecular imaging. Our group has reported fabricated iron oxide nanoparticles conjugated with tumor ligands for targeted TAT tumor detection at the molecular level [10]. The results indicate that thermoacoustic molecular imaging with functionalized iron oxide nanoparticles may contribute to targeted and functional early cancer imaging. And modified iron oxide combined with suitable tumor markers may also be used as novel nanomaterials for targeted and guided cancer thermal therapy.

In this paper, we investigate the feasibility of using dextran-coated Fe₃O₄ magnetic nanoparticles as a contrast agent in thermoacoustic tomography for hepatocellular carcinoma detection. Dextran-coated Fe₃O₄ magnetic nanoparticles administered intravenously would be phagocytosed by Kupffer cells, which are mainly settled in the liver. Furthermore, Kupffer cells will be redistributed when tumor occurs in the liver, and will absent or markedly decrease in number in tumor entity [11-16]. Therefore, liver tumor retains thermoacoustic signal intensity after dextran-coated Fe₃O₄ magnetic nanoparticles administration, while signal enhancement in normal liver tissue. This provides the improvement of lesion-to-liver contrast for thermoacoustic tomography. A fast thermoacoustic computed tomography system with a multielement linear transducer array was used to image cancerous liver tissue with circular scanning. The results show that the system can provide molecular imaging with functionalized contrast agents for high-contrast detecting hepatocellular carcinoma and has the potential to become a novel approach for used in clinical diagnosis in the future.

2. Data-acquisition system and imaging reconstruction
The data-acquisition system for TAT molecular imaging mainly composes of B-mode digital ultrasound diagnostic equipment (Model CTS-5000B, SIUI, China) with a multi-element transducer (L2L50A) and a high speed digital card (PCI-6541, NI, USA). The 128-element linear transducer with a scanning width of 53 mm was used for thermoacoustic signal acquisition. The multi-element transducer has a center frequency of 2.5 MHz with a nominal bandwidth of 70%. The transducer array, which has a computed tomography capability, can select the thermoacoustic signals which are parallel to its centre cross section in a limited view. The digital data acquisition (DAS) card featured a 12-bit analog-to-digital (D/A) converter with a sampling rate of 25 MHz was employed for collecting digital
signals. A microwave generator at 6 GHz (BW-6000HPT, China) transmitted out short microwave pulses adjustable from 0.3 to 1.2 µs, whose maximum trigger repetition rate can reach 500 Hz. The microwave pulses were coupled into a rectangular waveguide in TE10 mode with a cross section of 34.8 × 15.8 mm². For 6 GHz microwaves, the penetration depths of fat and muscle are 5.2 and 0.7 cm respectively. Other soft tissue has a penetration depth between those of fat and muscle. The energy density per volume of the microwave pulse is about 0.25 mJ/cm³, which is within the safety standard for animal and human use.

A custom-built control circuit provided a synchronized clock signal to trigger the DAS card and the microwave generator simultaneously. The TA signals received by the transducer array, after pre-amplification and phase adjustment, were acquired with the DAS card, and then transferred to a personal computer for subsequent data processing. The phantom in the holder can be rotated at several scanning positions for acquisition of multiple image signals with comprehensive tissue information. Generally the signals at 20 circular scanning positions around the object were sufficient to recover a clear and accurate image. Due to the directivity properties of the linear transducer array, an improved limited-field-filtered back-projection (LFBP) is employed to reconstruct the thermoacoustic image. More technical information about the fast data-acquisition system can be found in the previous literatures.

3. Results
Dextran-coated \( \text{Fe}_3\text{O}_4 \) magnetic nanoparticles supplied by Department of Biomedical Engineering, Zhejiang University are Fe\(_3\)O\(_4\) particles are almost spherical with diameters ranging from 10 to 20 nm. After coate with dextran, dextran-coated Fe\(_3\)O\(_4\) magnetic nanoparticles are almost spherical with the Fe\(_3\)O\(_4\) particles are almost spherical with diameters ranging from 10 to 20 nm. After coate with dextran, dextran-coated Fe\(_3\)O\(_4\) magnetic nanoparticles are almost spherical with diameters ranging from 30-50 nm[17].

Dextran-coated \( \text{Fe}_3\text{O}_4 \) magnetic nanoparticles for TAT molecular imaging. A stable water-soluble dextran-coated \( \text{Fe}_3\text{O}_4 \) magnetic nanoparticles suspension was obtained after the final centrifugation of the solution. In a phantom study, TA signals of dextran-coated \( \text{Fe}_3\text{O}_4 \) magnetic nanoparticles at different concentrations as shown in the Eppendorf tubes from left to right in the actual inset picture.
are detected under the same microwave energy density. It can be seen that the TA signal produced by dextran-coated Fe₃O₄ magnetic nanoparticles solution is in linear relationship with their concentrations ($R^2 = 0.9961$) in Fig. 2. These data suggest that TA signal of the contrast agent is much higher than water and dextran-coated Fe₃O₄ magnetic nanoparticles solution at a concentration of 0.1 mg/ml can achieve effective signal increase. The concentration at the level of 0.1 mg/ml can be used as a reference for the in vivo study.

![Graph showing linear fitting of thermoacoustic signals of contrast agents at different concentrations](image)

**FIG.2.** Linear fitting of thermoacoustic signals of contrast agents at different concentrations; the solid line is a linear fit with standard deviations (n=3). Dextran-coated Fe₃O₄ magnetic nanoparticles at different concentrations are shown in the Eppendorf tubes from left to right in the actual inset picture of Fig. 2. The thermoacoustic signal produced by dextran-coated Fe₃O₄ magnetic nanoparticles was observed to be linearly dependent on the concentration ($R^2 = 0.9965$).

Different concentrations of dextran-coated Fe₃O₄ magnetic nanoparticles solution mixed with human blood were also investigated to estimate the appropriate concentration for TAT molecular imaging. Four identical plastic tubes with a diameter of 1 mm filled by human blood and dextran-coated Fe₃O₄ magnetic nanoparticles solution were embedded at a depth of 10 mm in porky fat tissue, the cross section of the three tubes’ placement is shown in the inset picture of Fig. 3(a). The tube marked ‘A’ was filled by 150 µl dextran-coated Fe₃O₄ magnetic nanoparticles of 0.1 mg/ml with 150 µl blood. ‘B’ was filled by 150 µl dextran-coated Fe₃O₄ magnetic nanoparticles solution of 0.075 mg/ml mixed with 150 µl blood, another one marked ‘C’ was filled by 150 µl dextran-coated Fe₃O₄ magnetic nanoparticles solution of 0.05 mg/ml mixed with 150 µl blood; and the other one filled by 300 µl pure blood. The TAT image in Fig. 3(a) obtained by circular scanning at 20 scanning positions agrees well with the actual phantom shown in the inset of Fig. 3(a). The thermoacoustic map of tube ‘A’ containing dextran-coated Fe₃O₄ magnetic nanoparticles and blood has a much higher signal-noise-ratio (SNR) than that of tube ‘D’ containing blood only due to high microwave absorption of the exogenous contrast agent. Thermoacoustic signals were calculated in the tubes whose boundaries were clearly visualized in the ellipses, and shown in Fig. 3(b). The TA signals can be
increased roughly 4 times using a dextran-coated Fe₃O₄ magnetic nanoparticles enhanced sample compared to pure blood.

![Graph](image)

Figure 3. (a) Thermoacoustic imaging of thermoacoustic contrast agents mixed with human blood at four different concentrations in plastic tubes. (b) Thermoacoustic signals were calculated in the tubes whose boundaries were clearly visualized in the dashed ellipses. Data are the mean values of three replicates.

Because dextran-coated Fe₃O₄ magnetic nanoparticles administered intravenously would be phagocytosed by Kupffer cells, which are mainly settled in the liver. Furthermore, Kupffer cells will be redistributed when tumor occurs in the liver, and will absent or markedly decrease in number in tumor entity. Therefore, liver tumor retains thermoacoustic signal intensity after dextran-coated Fe₃O₄ magnetic nanoparticles administration, while signal enhancement in normal liver tissue. A phantom made from agar simulation the distribution of dextran-coated Fe₃O₄ magnetic nanoparticles in the liver with hepatocellular carcinoma after intravenous. the round phantom was made from 15% gelatin, 12.5% milk, and 72.5% water to simulate the electromagnetic and acoustic properties of live tissue. dextran-coated Fe₃O₄ magnetic nanoparticles were doped in the phantom but absent in a small round which simulation the tumor entity. The small round is in the middle of the big round phantom. Induced thermoacoustic waves emanated from the distribution of the water and the dextran-coated Fe₃O₄ magnetic nanoparticles, the signals at 20 scanning stops around the sample were acquired to reconstruct the images. Fig.4. shows the thermoacoustic image by the LFBP reconstruction algorithm with the real picture shown in the inset of Fig.4. This image clearly show that the signal from dextran-coated Fe₃O₄ magnetic nanoparticles is much higher than the water. we can see in the TAT image, the signal only enhance in distribution of the dextran-coated Fe₃O₄ magnetic nanoparticles, this result indicate that when dextran-coated Fe₃O₄ magnetic nanoparticles administered intravenously, and after phagocytosed by Kupffer cells, the signal from normal live tissue would be much higher than the tumor entity because Kupffer cells are mainly settled in the liver but absent in tumor entity. This result is successful ex vitro evidence for the possibility of application of dextran-coated Fe₃O₄ magnetic nanoparticles as thermoacoustic image for hepatocellular carcinoma detection.
FIG.4. Thermoacoustic imaging of a phantom made from agar simulation the distribution of dextran-coated Fe$_3$O$_4$ magnetic nanoparticles in the liver with hepatocellular carcinoma. The inset picture is photography of the simulated agar. Dextran-coated Fe$_3$O$_4$ magnetic nanoparticles only distribution in the ring of the big round simulated the normal live tissue and absent in the small round simulated the tumor entity.

4. Conclusion
In summary, we have successfully shown that the peak-to-peak TA signal can be increased roughly 4 times using a dextran-coated Fe$_3$O$_4$ magnetic nanoparticles enhanced sample compared to pure blood. The phantom Simulation the distribution of the liver with hepatocellular carcinoma after intravenous dextran-coated Fe$_3$O$_4$ magnetic nanoparticles clearly show that the signal from dextran-coated Fe$_3$O$_4$ magnetic nanoparticles is much higher than the water. This result indicate when dextran-coated Fe$_3$O$_4$ magnetic nanoparticles administered intravenously and after phagocytosed by Kupffer cells, the signal from normal live tissue would be much higher than the tumor entity because Kupffer cells are mainly settled in the liver but absent in tumor entity. We presented our preliminary results that the feasibility of using dextran-coated Fe$_3$O$_4$ magnetic nanoparticles as a contrast agent in thermoacoustic tomography for hepatocellular carcinoma detection and has the potential to become a novel approach for used in clinical diagnosis in the future.

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