Histology mimicking phantoms for the high-frequency ultrasound of breast cancer surgical margins: Comparison between gelatin and agarose media

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Abstract

At Utah Valley University, a high-frequency (HF) ultrasonic method has shown promise as a rapid, intraoperative method for detecting residual breast cancer in margins resulting from breast conservation surgery (BCS). Due to the difficulty of obtaining and routinely testing human tissue samples in a laboratory setting, soft tissue phantoms have been developed with inclusions to simulate the microstructures and histology of breast tissue pathologies. The objective of this study was to determine the optimal phantom medium to maximize the fidelity of the phantoms to actual breast tissue and its histology. Gelatin based phantoms were made from a mixture of distilled water, Knox gelatin, and Metamucil fiber while agarose based phantoms were created from distilled water, agarose powder, and TBE stock solution. In both phantom mediums polyethylene microspheres were embedded in layers in order to simulate breast tissue microstructure. Microsphere diameter varied by phantom while a constant volume percent was maintained. Pitch-catch measurements were acquired using 50-MHz transducers, a HF pulser-receiver, a 1-GHz digital oscilloscope, and glycerol as the coupling agent. Results from the gelatin phantoms showed a decrease in both peak density and attenuation values with increasing microsphere diameter and overall decreasing heterogeneity of the phantoms. The agarose phantoms showed the same results with comparable standard deviations. These results not only indicate that both gelatin and agarose based phantoms can be effectively used to accurately simulate breast tissue microstructure and pathology, but that the optimal phantom medium may be based on personal preference or experimental needs.

Keywords: High-frequency ultrasound, breast cancer
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1 Introduction

Breast Cancer is the most common cancer affecting women in the United States [1] and worldwide (excluding skin cancer) [2]. According to the United States’ Center for Disease Control and Prevention and the National Cancer Institute, approximately 230,000 new cases are recorded yearly in the United States, with roughly 40,000 of those cases resulting in death [3]. Statistically 60-75% of breast cancer patients opt to undergo breast conservations surgery (BCS) as their preferred method of treatment. BCS has many advantages that make it an appealing treatment plan for many breast cancer patients. BCS preserves breast tissue unaffected by the cancer, is far less invasive than other treatment options, including mastectomy, and has proven to be an equally effective treatment option for patients when accompanied with radiation therapy [4]. However, BCS also faces one significant disadvantage: 20-60% of breast cancer patients who undergo BCS will require a second or even third surgery to remove residual cancer left in the breast after the initial surgery [4,5]. Under current protocols surgeons send excised tissue from BCS to a pathologist for analysis. The excised tissue is fixed, sliced, and mounted onto slides for examination under a microscope. Thorough inspection of the prepared slides is conducted to ensure that the surgeon effectively removed all of the cancer and obtained clean margins surrounding the site of the excised tumor. This process is extensive and may take several days. As a result, many patients are released from the hospital and return home before pathology results regarding the status of their surgical margins are ever available. If cancer is found in the surgical margins most patients must return for additional surgery. Not only is a consecutive surgery costly, it subjects the patient to increased pain, discomfort, and emotional hardship. Therefore, an intraoperative analysis device used by the surgeon to test marginal tissue for residual cancer during the initial surgery would help reduce re-excision rates and improve patient outcomes.

A 2010 pilot study, conducted collaboratively between Utah State University and the Huntsman Cancer Institute in Salt Lake City, Utah, tested the possibility of the use of high-frequency (HF) ultrasound as a rapid intraoperative margin assessment tool. The 17-patient pilot study tested 53 positions over 34 excised surgical specimens from BCS operations. Results from the pilot study showed that the HF ultrasound parameters of spectral peak density and attenuation could be used to differentiate between normal, benign, and malignant breast tissue pathologies [6]. A second 73-patient follow-up study was conducted in 2014 by Utah Valley University and the Huntsman Cancer Institute. This study tested 492 BCS surgical specimens and 1112 positions, of which 722 were on margins. The results of the follow-up study corroborated those of the pilot study and demonstrated the sensitivity of peak density and attenuation to breast tissue pathologies. Results of the follow-up study showed that peak density could differentiate malignant from nonmalignant pathologies with an accuracy 71.1% (margins) to 79.5% (lymph
Trends from the pilot study closely resemble the 2014 study's results, with an accuracy of 71.7% for peak density. Application of these trends to the follow-up study predicts that a multivariate analysis will yield much higher accuracy (84.1%), specificity (85.2%), and sensitivity (77.6%) values [7].

In order to interpret and better understand these results, laboratory experiments must be conducted. In ultrasound laboratory experiments, much of the time it is not possible to use the actual tissues in which the technique will be applied. For this reason, it is necessary to simulate these tissues while maintaining the characteristics of the expected phenomenon through tissue phantoms [8]. It is important to use materials when constructing ultrasound phantoms that have the same ranges of speed sound, attenuation, and scattering properties which one is trying to mimic [9]. As a result, many different phantom mediums are available for studying the same tissue such as breast tissue. The purpose of this study was to explore the effects of different phantom matrix materials for human breast tissue in ultrasonic studies, to incorporate polyethylene microspheres of varying sizes and concentrations to mimic differing breast tissue pathologies, and to provide results that will help improve and maximize the analysis and interpretation of surgical study data.

2 Methods

2.1 Phantom Preparation

In the first phantom experiment, breast tissue phantoms were created using a mixture of unflavored gelatin (Knox brand gelatin) and sugar-free psyllium hydrophilic muciloid fiber (Metamucil fiber). This combination of Knox gelatin and Metamucil fiber was selected as the phantom medium due to its ability to mimic the parenchymal tissues of the breast. In addition, gelatin phantoms made with psyllium hydrophilic muciloid fiber can be prepared much more easily, more rapidly, and with more reproducibility than other gelatin materials [10]. Ingredients in the following proportions were used to make the phantoms: one cup boiling water, three packets of Knox brand gelatin, and approximately one tablespoon sugar-free Metamucil. All ingredients were stirred together until completely dissolved [10]. Polyethylene microspheres (Cospheric) were incorporated into each phantom in order to mimic varying breast tissue pathologies. A calculated 0.2165 g of each microsphere size, 58-550 μm in diameter, was measured out in order to produce phantoms of variable microsphere diameter by sample and constant volume percent. Each measured quantity of polyethylene microspheres was divided into three equal proportions for inclusion in the phantoms. The microsphere inclusions were added to the phantoms in layers (3-4 mm thick), in order to eliminate the production of air bubbles, to prevent microspheres from settling, and to ensure proper distribution of the microspheres within the phantoms. A first layer of the Knox gelatin and Metamucil fiber mixture was poured into a molding tray and polyethylene microspheres were gently mixed into the layer until evenly distributed. In order to not disturb the previous layer, the base layers were allowed to cool and solidify before consecutive layers were added. Upon solidification, a successive layer of with polyethylene bead inclusions was carefully added. This process was continued for the following layer before a final layer of gelatin, without microsphere inclusions, was added to
act as a coupling layer during ultrasound testing. Each completed phantom totaled approximately 16 mm thick. Three 16 mm phantoms without microsphere inclusions were also produced as controls in this experiment. Figure 1 is a photograph of the completed gelatin phantoms with red polyethylene microsphere inclusions.

In a second phantom experiment breast tissue phantoms were created using 10X TBE stock solution, distilled water, and agarose powder to create 3% agarose phantoms by concentration. Agarose was selected as the phantom medium as a result of its well-characterized performance, the ease of fabrication, and the flexibility to incorporate additional ingredients to achieve a range of target acoustic properties [11]. The agarose medium phantoms were made in a like manner to the gelatin phantoms. Polyethylene microspheres (Cospheric) were incorporated into each phantom in order to mimic varying breast tissue pathologies. A calculated 0.2738 g of each microsphere size, 58-550 μm in diameter, was measured out in order to produce phantoms of variable microsphere diameter by sample and constant volume percent (0.08%). Each measured quantity of polyethylene microspheres was divided into four equal proportions for inclusion in the phantoms. The microsphere inclusions were added to the phantoms in layers (4 mm thick), in order to eliminate the production of air bubble and ensure proper distribution of the microspheres within the phantoms. A first layer of agarose was poured into a molding tray and polyethylene microspheres were gently mixed into the layer until evenly distributed. In order to not disturb the previous layer, the base layers were allowed to cool and solidify before consecutive layers were added. Upon solidification, a successive layer of agarose with polyethylene bead inclusions was carefully added. This process was continued for the following two layers before a final layer of agarose, without microsphere inclusions, was added to act as a coupling layer. Each completed phantom totaled approximately 20 mm thick. Three 20-mm phantoms of 3% agarose without microsphere inclusions were also produced as
controls in this experiment. Figure 2 is a photograph of the completed agarose phantoms with red polyethylene microsphere inclusions.

![Completed agarose phantoms with red polyethylene microsphere inclusions.](image)

**Figure 2:** Completed agarose phantoms with red polyethylene microsphere inclusions of variable diameters by sample and constant volume percent. 1-3 are controls containing no microsphere inclusions. By microsphere size: 4) 53 μm, 5) 90 μm, 6) 180 μm, 7) 355 μm, and 8) 500 μm.

### 2.2 Phantom Testing

Pitch-catch (through-transmission) and pulse-echo measurements were acquired with the use of two 50-MHz immersion transducers (Olympus NDT, V358-SU, 12.7-mm OD, 6.35-mm active element diameter), a HF square-wave pulser/receiver (UTEX, UT340), and a 1-GHz (Agilent, DSOX3104A) digital oscilloscope. An aluminum test fixture supported the phantom specimens and held the transducers in contact with the phantoms (Figure 3). Ultrasonic waveforms were averaged in the signal acquisition and specimen thickness was recorded for each measurement. During ultrasonic testing, each specimen was placed inside a resealable plastic storage bag to protect the phantom from contamination or damage, and the outside of the bag was coupled to the transducers with glycerol. Three to four positions were tested on each phantom based on phantom size. Triplicate waveforms were recorded for each test position.

Peak densities were derived from the time-domain waveforms by performing a Fourier transform and counting the number of peaks and valleys in the 20-80 MHz region of the resulting power spectra. Attenuations were calculated by scaling the waveforms to account for receiver gain and tissue thickness, and using a Hilbert transform to obtain the waveform envelopes and their corresponding amplitudes.
3 Results

3.1 Phantom Experiments

Data from both the gelatin and agarose experiments showed that both peak density and attenuation varied with microsphere diameter. However, peak density followed an inverse particle size relationship [Figures 4(a) and 5(a)], whereas attenuation increased linearly as microsphere diameter decreased [Figures 4(b) and 5(b)]. The experiments suggest that peak density arises principally from scattering at the microscopic level, whereas attenuation is sensitive to the concentration of scatterers present. The data also suggests that peak density is also independent of specimen thickness, further demonstrating that it is sensitive mainly to scatterer size. The attenuation results are most likely due to an increase in inclusion concentration (as inclusion size decreases, inclusion number increases for a fixed volume of inclusions).
Figure 4: a) Peak density results for gelatin based phantoms, showing peak density increases with smaller sized scatterers. b) Attenuation results for gelatin based phantoms, showing attenuation values increase with larger numbers of scatterers present.

Figure 5: a) Peak density results of agarose based phantoms, corroborating those of the gelatin phantoms and showing peak density increases with smaller sized scatterers. b) Attenuation results of agarose based phantoms, again showing attenuation values increase with larger numbers of scatterers present.

3.2 Comparison of Results

The results of both the gelatin and agarose phantoms experiments corroborate one another. Results from both phantom media showed a decrease in both peak density and attenuation values with increasing microsphere diameter and overall decreasing heterogeneity of the phantoms. The agarose and gelatin phantoms overall showed the same results along with markedly comparable standard deviations. These results indicate that both gelatin and agarose
based phantoms can be effectively used to accurately simulate breast tissue microstructure and pathology.

4 Conclusions

Breast cancer is the most common cancer affecting women in the United States [1] and worldwide (excluding skin cancer) [2]. Approximately 60-75% of diagnosed breast cancer patients choose to undergo breast conservations surgery as their preferred method of treatment. Considering current re-excision rates for patients that undergo BCS, the development of an intraoperative margin assessment tool is of high priority. A 2010 pilot study and a consecutive 2014 follow-up study showed that specific HF ultrasound parameters are sensitive to breast tissue pathologies making HF ultrasound a candidate technology of margin assessment. In order to interpret the surgical data and improve, experiments must be conducted in the laboratory using tissue phantoms. Many phantom medias are available for purchase or fabrication but it is vital that the optimal media is used. The purpose of this research was to determine the most favorable phantom media for mimicking breast tissue and its pathologies under HF ultrasound. Studies were conducted using both gelatin and agarose based phantoms. Polyethylene microspheres were embedded into the phantoms in order to simulate breast tissue pathologies. Both the gelatin and agarose phantom experiments showed larger peak density values with smaller diameter bead inclusions and larger attenuation values with greater concentrations of scatterers present. Agarose and gelatin phantom media showed similar standard deviations. These results indicate that both gelatin and agarose based phantoms can be used to simulate breast tissue and that the optimal phantom medium may be based on personal preference or experimental needs.

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