Radiation Physics and Chemistry 90 (2013) 67–72

Contents lists available at SciVerse ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

X-ray scattering for the characterization of lyophilized breast tissue samples

Wael M. Elshemey*, Fayrouz S. Mohamed, Ibrahim M. Khater

Biophysics Department, Faculty of Science, Cairo University, Giza, Egypt

HIGHLIGHTS

- X-ray scattering profiles of breast tissue samples are acquired.
- Three X-ray profile characterization parameters are calculated.
- The cut-offs, sensitivity, specificity and diagnostic accuracy are calculated.
- They are compared to the data from non-lyophilized samples.
- Results show increased sensitivity in case of lyophilized samples.

ARTICLE INFO

Article history:
Received 10 December 2012
Accepted 21 April 2013
Available online 30 April 2013

Keywords:
X-ray scattering
Breast cancer
Lyophilized tissue
Tissue characterization

ABSTRACT

This work investigates the possibility of characterizing breast cancer by measuring the X-ray scattering profiles of lyophilized excised breast tissue samples. Since X-ray scattering from water-rich tissue is dominated by scattering from water, the removal of water by lyophilization would enhance the characterization process. In the present study, X-ray scattering profiles of 22 normal, 22 malignant and 10 benign breast tissue samples are measured. The cut-offs of scatter diagrams, sensitivity, specificity and diagnostic accuracy of three characterization parameters (full width at half maximum (FWHM) for the peak at 1.1 nm^{-1}, area under curve (AUC), and ratio of 1st to 2nd scattering peak intensities (I_1/I_2%)) are calculated and compared to the data from non-lyophilized samples. Results show increased sensitivity (up to 100%) of the present data on lyophilized breast tissue samples compared to previously reported data for non-lyophilized samples while the specificity (up to 95.4%), diagnostic accuracy (up to 95.4%) and receiver operating characteristic (ROC) curve values (up to 0.9979) for both sets of data are comparable. The present study shows significant differences between normal samples and each of malignant and benign samples. Only subtle differences exist between malignant and benign lyophilized breast tissue samples where FWHM$=0.7\pm0.1$ and 0.8 ± 0.3, AUC$=1.3\pm0.2$ and 1.4 ± 0.2 and I_1/I_2%$=44.9\pm11.0$ and 52.4 ± 7.6 for malignant and benign samples respectively.

1. Introduction

There are two pathological techniques used in the process of breast tissue diagnosis, the first one is the frozen section technique in which fresh tissue is examined during breast surgery in order to allow the surgeons to take an immediate therapeutic decision. The second technique is the paraffin section (permanent section) technique that takes place after the surgery where the sample preparation for this examination takes long time. This technique is more accurate than the frozen section technique so it is used to confirm the results of the frozen section technique. Both of the previously mentioned techniques require experience and knowledge of clinical medicine and pathology. Moreover, chemical sample preparations are required to examine the sample using these methods (Fergenbaum et al., 2004; Karve et al., 2005; Sultana and Kayani, 2005). Using X-ray scattering to characterize breast tissue samples has the advantage that it does not depend on personal experience or knowledge of clinical medicine and pathology. Moreover, no chemical sample preparations are required to examine the sample using this technique. Therefore, X-ray scattering technique would be helpful as a confirmatory test for the results of the pathology tests.

Several studies have shown that it would be possible to characterize excised normal and malignant breast tissue samples through their X-ray scattering profiles. The characterization is based on the presence of higher fat content in normal breast tissue compared to malignant tissue (Kidane et al., 1999; Ryan and Farquharson, 2004;

* Corresponding author. Tel.: +20 1001560029.
E-mail address: waelelshemey@yahoo.com (W.M. Elshemey).

0969-806X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.radphyschem.2013.04.019

Farquharson and Geraki, 2004, Changizi et al., 2005; Elshemey and Elsharkawy, 2009; Cunha et al., 2011). This is reflected in the distinctive features of the sharp fat peak at 1.1 nm^{-1} compared to the soft tissue peak at 1.6 nm^{-1} in normal and malignant tissues respectively (Kidane et al., 1999; Castro et al., 2004; Ryan and Farquharson, 2007). The available studies in this field are all performed using unprocessed excised breast tissue samples (Cunha et al., 2006; Griffiths et al., 2007; Oliveira et al., 2008; Changizi et al., 2006; Elshemey et al., 2010a; Pani et al., 2010; Conceição et al., 2011). The samples are thus of high water content. This would probably affect the characterization process since the scattering of X-rays from water-rich biological samples is dominated by the scattering from water (Kosanetzky et al., 1987). Lyophilization yields X-ray scattering profiles that unveil characteristic peaks for different biological samples. While most biological samples show a diffraction peak at d-spacing=4.5 Å (equivalent to momentum transfer value of 1.1 nm^{-1}), protein-rich samples are characterized by an additional scattering peak at d-spacing of 10 Å (equivalent to momentum transfer value of 0.5 nm^{-1}). The scattering peak from fat is sharper and more intense compared to the other biological samples (Desouky et al., 2001). In protein, the peak at momentum transfer value of 1.1 nm^{-1} (d-spacing of 4.5 Å) is attributed to the hydrogen bonding spacing in the backbone of protein secondary structure while the peak at momentum transfer value of 0.5 nm^{-1} (d-spacing of 10 Å) corresponds to the spaces between alpha helices (or beta sheets) and its amplitude corresponds to the content of such protein secondary structure (Bouchard et al., 2000; Peng et al., 2004; Kreplak et al., 2004; Ying et al., 2005; Serefoglou et al., 2008; Elshemey et al., 2010b). X-ray scattering profiles have been successfully used to characterize several lyophilized biological samples, including blood, fat, DNA, muscle and proteins (Elshemey et al., 2001; Desouky et al., 2001). In this work, 22 normal, 22 malignant and 10 benign excised breast tissues samples are lyophilized and their X-ray scattering profiles are acquired. Three X-ray scattering profile characterization parameters are measured (full width at half maximum (FWHM) for the peak at 1.1 nm^{-1}, area under curve (AUC), and ratio of 1st to 2nd scattering peak intensities (I_1/I_2%)). The cut-offs of dot diagrams, sensitivity, specificity and diagnostic accuracy are calculated and receiver operating characteristic (ROC) curves are plotted. The benign samples are measured in order to examine the possibility of distinguishing between malignant and benign tumors in lyophilized samples. Since the process of lyophilization takes time, this technique is expected to offer a confirmatory tool for the results of the paraffin section technique of breast tissue diagnosis.

2. Materials and methods

Fifty-four excised breast tissue samples are collected over a period of about 1 year from women undergoing mastectomy and preserved in formalin until lyophilized using LABCONC freeze dryer, Kansas City, USA. Freeze drying, also called lyophilization, is the process of removing water from frozen samples held in a vacuum chamber. The frozen water is converted directly into water vapor without an intermediate stage involving liquid water. It is a non-destructive method for preserving biological samples. It can preserve even viable samples of viruses and certain microorganisms, such as yeast. During lyophilization, samples are held at about −40 °C in a vacuum of a maximum of 0.1 mmHg.

Although the breast tissue samples used in the present study are of nearly equal volume and are lyophilized at a fixed time interval of 48 h, it is expected that minor variations would exist in the amount of water removed from samples of the same tissue type. These possible variations are included in the standard deviation listed in Table 1 for each tissue type for the different characterization parameters. Preservation of breast tissue samples in formalin has been reported not to affect their X-ray scattering profiles (Peplow and Verghese, 1998; Castro et al., 2004). Formalin is used only to preserve samples until lyophilized. In practice, an excised breast tissue sample can be directly lyophilized and measured after surgery. Samples are classified by a histopathologist into normal (including healthy breast tissue surrounding the pathological tissue), malignant and benign breast tissue samples.

X-ray scattering data of lyophilized breast tissue samples are acquired using a Shimadzu XRD-6000 X-ray diffractometer working at 40 kV and 30 mA in reflection geometry (θ–2θ linkage mode). The diffractometer has a high-precision, vertical goniometer with a high-speed rate (up to 1000 deg/min) and high-precision angle reproducibility ($\pm 0.001^\circ$). It has a 1° divergence slit, a 1° scatter slit and a 0.30 mm receiving slit. The device employs a Cu target and a graphite monochromator in order to produce 8.047 keV collimated X-ray beam. Measurements are carried out from $2\theta=4^\circ$ to 70° in a step mode at a step equal to 0.25° and a total collection time of 13 min per scan. Diffraction data are collected using a scintillation detector employing a sodium iodide crystal. A metal sample holder with a rectangular aperture of an area 2×1.7 cm^2 and a thickness of 0.1 cm is used to hold the sample which fills the space in the direction of the beam with special care that the sample totally lies in the same plane of the holder.

The collected data are smoothed using three-point averaging, and peak normalized at the scattering peak of 1.1 nm^{-1}. No additional data corrections or tail fitting are used. Three X-ray scattering profile characterization parameters are calculated. The FWHM parameter is calculated for the peak at 1.1 nm^{-1} by drawing the base line of the peak and measuring the width of the normalized peak in nm^{-1} at half the distance between the peak tip and the baseline (Fig. 1). The I_1/I_2% parameter is calculated by dividing the y-axis value of the normalized peak at 0.55 nm^{-1} (0.42 nm^{-1} for malignant and benign samples) by that at 1.1 nm^{-1}. The AUC parameter is calculated by numerical integration of the area under the normalized X-ray scattering profile using Origin 6.0 software analysis tool.

The optimal cut-off values for differentiating between normal and each of malignant and benign samples in the scatter diagrams are calculated as mean+2SD of the normal samples (Hada et al., 1999; Verma et al., 2010) while the optimal cut-offs for differentiating between malignant and benign samples are simply the means (not mean+2SD) of malignant samples. The choice of the optimal cut-off is discussed in more detail in the Section 3.

In order to calculate the sensitivity, specificity and diagnostic accuracy of each of the investigated characterization parameters we have to remember that, true positive samples are diseased samples whose characterization parameter values lay above the cut-off while false positive samples are normal samples having values above the cut-off. Similarly, true negative samples are normal samples lying below the cut-off and false negative samples are diseased samples lying below the cut-off.

The sensitivity of a diagnostic test is the percentage of the samples with the disease which are correctly identified by the test as positive (true positive divided by true positive+false negative). The specificity of a diagnostic test is the percentage of the samples without the disease which are correctly identified by the test as

Table 1
Mean values of characterization parameters ± the standard deviation of the mean.

	Normal	Malignant	Benign
FWHM (nm^{-1})	0.4 ± 0.0	0.7 ± 0.1	0.8 ± 0.3
Area under curve	0.9 ± 0.1	1.3 ± 0.2	1.4 ± 0.2
(I_1/I_2%)	22.4 ± 4.3	44.9 ± 11.0	52.4 ± 7.6