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Preliminary study of human breast tissue using synchrotron radiation combining WAXS and SAXS techniques

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A B S T R A C T

Using synchrotron radiation, we combined simultaneously wide angle X-ray scattering (WAXS) and small angle X-ray scattering (SAXS) techniques to obtain the scattering profiles of normal and neoplastic breast tissu~es samples at the momentum transfer range $6.28\,nm^{-1} \le Q(=4\pi \cdot \sin(\theta/2)/\lambda) \le 50.26\,nm^{-1}$ and $0.15\,nm^{-1} \le Q \le 1.90\,nm^{-1}$, respectively. The results obtained show considerable differences between the scattering profiles of these tissues. We verified that the combination of some parameters (ratio between glandular and adipose peak intensity and third-order axial peak intensity) extracted from scattering profiles can be used for identifying breast cancer.

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1. Introduction

Breast cancer is the most frequently incident in women and accounts for almost 20% of all cancer deaths (Rogers et al., 1999). Mammography is the principal technique for early detection of breast cancer, but it is neither 100% sensitive nor 100% specific, presenting both false negative and false positive diagnoses and leading to undiagnosed cancer and inappropriate biopsies. This fact is mainly due to small differences in attenuation properties of the breast tissues (Kidane et al., 1999; Tomal et al., 2008) and the non-ideal observer performance. Several complementary techniques have been introduced in order to eliminate these inherent limitations, as for example sonography and magnetic resonance imaging (MRI) correlations, tomosynthesis, phase-contrast imaging. On the other hand, recent investigations have shown that X-ray scattering analysis could be used for early detection and diagnosis of breast cancer (Kidane et al., 1999; Speller, 1999; Lewis et al., 2000; Poletti et al., 2002a; Fernández et al., 2005; Round et al., 2005; Cunha et al., 2006; Griffiths et al., 2007 and Oliveira et al., 2008), presenting values of sensitivity and specificity for WAXS of 95.6% and 82.3% for a correct differentiation between normal and neoplastic samples and 78.6% and 62.5% between benign and malignant samples, respectively, (Oliveira et al., 2008). While for SAXS, the best sensitivity and specificity values were 100% and 80% (Round et al., 2005). The motivation of these works lies in the important fact of intensity of the coherently scattered photons from breast tissues carry information related to the structural organization present in these tissues (Speller, 1999). Consequently, if cancer cells cause changes into the biological structures of tissues that they attack, this alteration will be present in the scattering profile (the distribution of the number of scattering photons as function of scattering angle or momentum transfer) of the tissue, being this distribution due to the collective scattering effects due to atom, molecule and supra molecular arrangements in the material. For a given energy, the scattering effects in high angles are due to the internal atomic structure of the material, therefore, the interferences among the several atoms that compose the tissue are not detected (Poletti et al., 2002b). In intermediate or wide angles, atomic or molecular interferences appear in the scattering profile, while in small angles this profile reveals information about supramolecular structures present into the tissues (Fernández et al., 2005). These effects of interferences cause a unique and characteristic scattering profile for each material (or tissue), which can be computed if the structural parameters are known (spatial distribution of charge, distance among atoms and so on). As these parameters are, in general, unknown a priori, the scattering profile should be experimentally measured.

Usually two techniques are used to measure scattering profiles from human breast tissues: WAXS (wide angle X-ray scattering) and SAXS (small angle X-ray scattering) (Theodorakou and Farquharson, 2008). WAXS technique allows obtaining a spatial distribution of smallest cell structures that compose the tissues, as for example water and fatty acid (Evans et al., 1991; Tartari et al., 1997; Kidane et al., 1999; Poletti et al., 2002a, c; Cunha et al., 2006; Griffiths et al., 2007; Oliveira et al., 2008). These works have shown that some parameters extracted from the scattering profile, as peak position, height and FWHM (Kidane et al., 1999), ratio of

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peaks intensities at 19.6 and 13.8 nm^{-1} (Griffiths et al., 2007) and the complete shape (Cunha et al., 2006; Oliveira et al., 2008), could be used to differentiate between healthy and neoplastic human breast tissue. On the other hand, SAXS technique allows determining supramolecular system features, for example the protein collagen, the major structural component of the extracellular matrix in breast tissues (Rogers et al., 1999; Lewis et al., 2000; Fernández et al., 2004). Lewis et al. (2000) extracted two parameters (intensity of third-order peak and third-order axial spacing) from the scattering profiles and showed that the values of these parameters are significantly different among normal tissue, benign and malignant diseases. Fernández et al. (2002, 2004) used the scattering profile to determine some characteristic parameters (axial period, average X-ray scattering intensity, fibril diameter and packing) and concluded the each type of tissues can be classified by them. Round et al. (2005) used the complete curve of scattering intensity with respect to the momentum transfer and reduced it using principal component analysis (PCA) in order to classify normal and diseased tissues. Falzon et al. (2006) applied wavelet decomposition to SAXS image and showed that successful identification of breast tissues malignancy can be achieved using wavelet coefficients and supervised classification.

In summary, WAXS and SAXS experiments independently can provide important parameters to detect and diagnose a disease (cancer). Therefore it is expected that the combination of both techniques simultaneously can correlate structural changes at atomic level with changes at larger scales (Ali et al., 2004; Tartari et al., 2005). It also allows several parameters be studied in a single experiments improving the diagnostic information present in the scattering profile. Therefore, in this preliminary study, both techniques were simultaneously used on each sample (normal and neoplastic breast tissue) to determinate the scattering profiles and to analyze what parameters can be used to classify different human breast tissue. The authors are unaware of any work in the literature combining WAXS and SAXS techniques in a single experiment to try to differentiate among healthy, benign and malignant human breast tissue.

2. Experimental systems and methods

2.1. Breast tissue samples

The breast tissue samples analyzed in this work were obtained from mastectomy and reduction mammoplasty procedures. After collected, they were histopathologically classified as normal tissues (comprising fibroglandular and adipose tissues), fibroadenomas (benign disease) or carcinomas (malignant disease). Subsequently to collection and classification, the samples were stored within suitable receptacles and fixed in formalin (4% formaldehyde in water). A total of 8 samples were analyzed in this preliminary work, being: three normal tissues, three carcinomas and two fibroadenomas. At the moment of the measurements, the samples were cut to 10 mm × 2 mm × 1 mm, inserted into a sample-holder and covered at both sides by thin mica foils.

2.2. Experimental setup

The combination of WAXS with SAXS was implemented in D11A–SAXS1 beamline at the National Synchrotron Light Laboratory (LNLS) in Campinas, Brazil. The experimental setup used in these experiments can be observed in Fig. 1. A focused monochromator of Si (111) was used in order to provide an X-ray beam of wavelength 1.48 Å and to reduce the irradiation area (1.0 mm × 0.5 mm) on the sample. The detectors for both techniques were two-dimension Fuji Bas III image plates. The WAXS image plate detector was fixed on a cylinder detector-holder focused on the sample and at a distance of 200 mm to the sample, as shown in Fig. 1, allowing a recording range of momentum transfer of $6.28\,nm^{-1} < Q(=4\pi \cdot \sin(\theta/2)/\lambda) < 50.26\,nm^{-1}$. The sample to SAXS image plate detector distance was fixed at 1.59 m and this space was evacuated using a vacuum chamber in order to minimize air scattering and absorption losses. A beam stop of 8 mm-diameter was inserted adjacent and centrally to detector in order to avoid the saturation of the detector produced by primary intensity. In this case the accessible momentum transfer range was $0.15\,nm^{-1} < Q < 1.90\,nm^{-1}$. Both image plates were read out and digitalized with a high resolution scanner (100 μm) MAR 300 of Molecular Dynamics®. The time of measurement was adjusted in order to assure statistical uncertainty smaller than 1% to the scattered photons intensity. Standard samples, alumina (Al_2O_3) and SilverBehenat ($CH_3(CH_2)_{20}COOAg$), were used as calibrant to WAXS and SAXS, respectively, in order to establish the correct reciprocal space scale of each pattern.

2.3. Data reduction

All digitalized images (standards, samples and sample-holder) of WAXS and SAXS were processed using the software Fit2D written by Andrew P. Hammersley, available by European Synchrotron Radiation Facility (Hammersley et al., 1996). From WAXS images, the distribution of scattered intensity as function of the momentum transfer was integrated at the vertical direction at the center of each strip, Fig. 2, while for SAXS images this integration was performed at both directions, meridian and equator, as is shown in Fig. 3 (Wilkinson et al., 2006). Several steps are necessary in order to obtain the linear differential scattering coefficient μ_S, which is defined as $\mu_S = n_V d\sigma/d\Omega$, being n_V the number of molecules per unit of volume and $d\sigma/d\Omega$ the total molecular differential cross-section. The first one is to correct the data for incident beam decay. Second, the number of photons originated from every other spurious scattering sources (sample-holder, collimators, etc.) must be subtracted from the original data. Third, the resulting data have to be corrected for a geometric

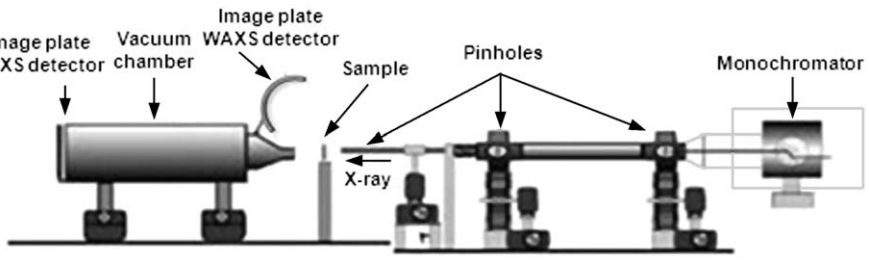

Fig. 1. Experimental setup.

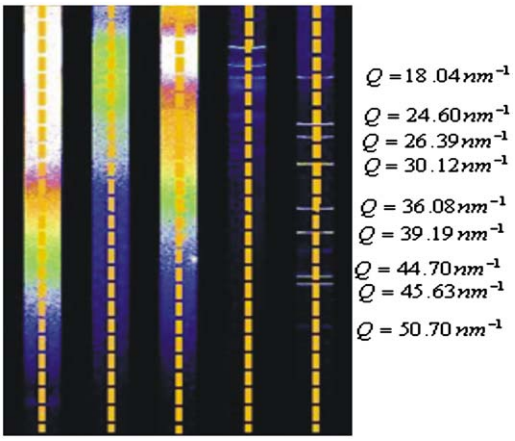

Fig. 2. WAXS images. From left to right, each strip represents: three types of breast tissue, background signal and standard calibration sample.

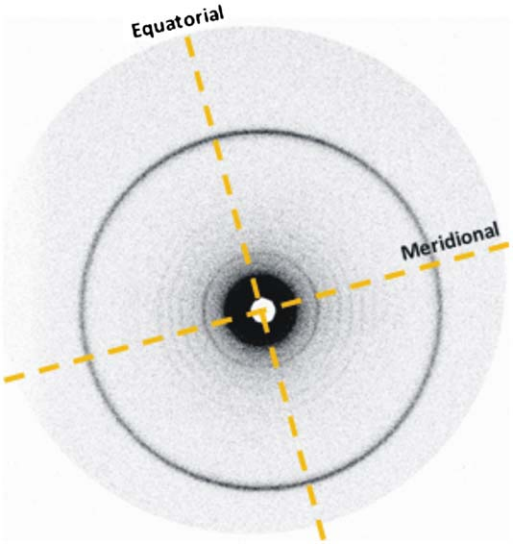

Fig. 3. Example of SAXS experimental image with system of coordination (meridional and equatorial axis) marked.

self-attenuation factor. For WAXS experiment, this latter factor presents exponential behavior, and is obtained by Monte Carlo methods, while, for SAXS experiment this factor can be considered a constant value for each sample data, given that $\cos(\theta) \approx 1$ (this factor corresponds to the transmission factor and it was measured at the moment of data collection). Finally, the last step consists in normalizing the data, by using a scaling factor, in order to obtain the absolute linear differential scattering cross section. The scaling factor is obtained from the ratio between theoretical, which can be obtained through IAM (Hubbell et al., 1975), and experimental values of μ_S at higher momentum transfer, where interferences effects do not occur (Poletti et al., 2002b). Denoting $I_{S+BG}(Q)$ and $I_{BG}(Q)$ as the total (sample and background) and background scattering intensity, corrected for incident beam decay, respectively, the scattering profile (μ_S) can be determined using

$$\mu_S(Q) = [I_{S+BG}(Q) \cdot A_{S+BG}(Q) - I_{BG}(Q) \cdot A_{BG}(Q)] \cdot C \quad (1)$$

where $A(Q)$ correspond to geometric self-attenuation factor; the indexes S and BG into the brackets correspond to sample and background, respectively, and C is the scaling factor.

3. Results and discussion

Figs. 4 and 5 show the mean scattering profiles for each group of tissues analyzed in this work for WAXS and SAXS, respectively. It should be note that the aim of this preliminary work was the identification of features that may be used to differentiate among normal, benign and malignant human breast tissue using WAXS and SAXS techniques simultaneously and not to perform a full molecular structure analysis of breast tissue.

Preliminary results based on the scattering profile obtained with WAXS technique show that all normal breast tissues have a first maximum peak at $Q = 13.9\,nm^{-1}$ (Tartari et al., 1997; Peplow and Verghese, 1998; Kidane et al., 1999; Poletti et al., 2002a, c), which is related to structures of fatty acid, principal component of adipose cells (Tartari et al., 1997). For benign and malignant tissues, the maximum peak appear in another position ($Q = 19.8\,nm^{-1}$), this peak position agreeing with previous works (Kidane et al., 1999; Griffiths et al., 2007; Oliveira et al., 2008) and being related probably to the high water-content in their constitution (Poletti et al., 2002a; Ryan and Farquharson, 2007). The only difference between benign and malignant tissues is the intensities of the peaks.

From SAXS scattering profiles, several peaks may be clearly seen in all samples studied, from 3rd ($Q = 0.29\,nm^{-1}$) to 12th ($Q = 1.17\,nm^{-1}$), corresponding to structures with a fundamental spacing of approximately 65 nm. This d-spacing is consequence of the staggered arrangement of tropocollagen within the fibrils (Bigi and Roveri, 1991; Lewis et al., 2000). Analyzing the scattering

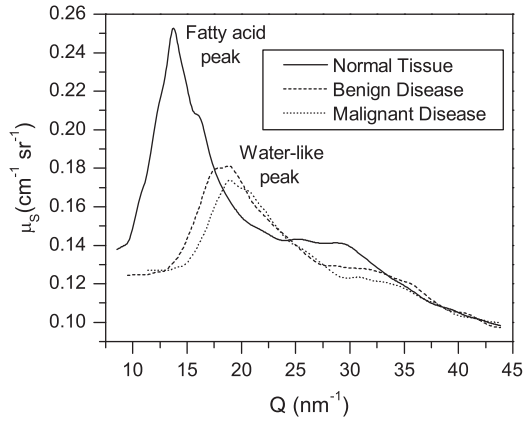

Fig. 4. Mean WAXS profile of each type of breast tissue analyzed in this work.

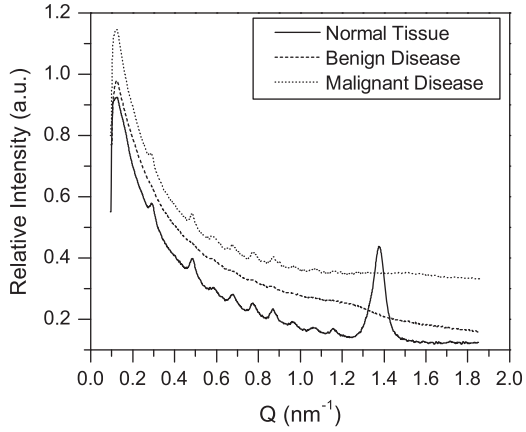

Fig. 5. Mean SAXS profiles of each type of breast tissue analyzed.

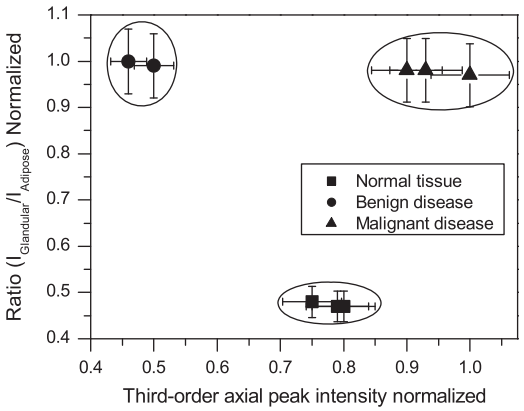

Fig. 6. Sample distribution on basis of combining of SAXS (third-axial order peak intensity) and WAXS (ratio of water-like and fatty acid peak intensities) parameters, with these uncertainties associated.

profiles shown in Fig. 5, it was observed that the intensity of third order axial peak was greater in malignant tissues than in other ones. This observation is in agreement with Pucci-Minafra et al. (1993), who pointed that this is related with an increase of the specific surface area of new fibril collagen induced by cancerous cells. Additionally in the normal tissues, another peak arises at $Q = 1.38\,\mathrm{nm}^{-1}$, corresponding to axial d-spacing characteristic of packing of triacylglycerols, presents in adipose cells (Bouwstra et al., 2001).

In order to verify if the differences found for each tissue type, combining WAXS and SAXS techniques, may be useful to characterize them, one parameter of each scattering profiles was extracted. For WAXS, the ratio between the intensity of water-like ($Q = 19.8\,\mathrm{nm}^{-1}$) and fatty acid ($Q = 13.9\,\mathrm{nm}^{-1}$) peaks (Griffiths et al., 2007) was used. For SAXS, the intensity of 3rd order axial peak (Lewis et al., 2000) was used. These parameters are shown in Fig. 6 together with their uncertainties. From Fig. 6, can be clearly observed the formation of clusters well-separated for each type of breast tissue analyzed in this work. Although few samples were used in this preliminary study, this finding suggests the possibility of using these parameters to try to differentiate among healthy, benign and malignant lesions, which encourages us to pursue further studies to confirm this finding.

4. Conclusion

X-ray scattering experiments applied in human breast tissues, healthy and neoplastic, provide a unique scattering profile to each tissues type, exhibiting features that allows distinguishing among normal, malignant and benign tissues. Using WAXS technique it is possible to find features at molecular level, fatty acid and water, for example, while changes in a supramolecular level, as collagen fibrils, can be observed employing SAXS technique. Combining both techniques allows correlate changes at molecular and supramolecular levels. Although it is yet beginning, this study shows that the combination of WAXS with SAXS provides a potential medical tool for characterizing small samples of human breast tissues.

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