



A synchrotron XRF study on trace elements and potassium in breast tissue

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Abstract

A synchrotron X-ray fluorescence system was employed to quantify the levels of iron, copper, zinc and potassium in 80 healthy and cancerous breast tissue samples. The statistical analysis of the sample concentrations reveals a trend of elevated levels in the cancerous tissue. The elevation is most pronounced for potassium and least for iron.

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1. Introduction

In recent years associations have been made between the levels of particular trace elements in human tissue and the presence of various diseases. It is in this same context that active interest continues to be shown in possible correlations between elemental concentrations and breast cancer. The involvement of some trace elements in the development of breast tumours has been suggested to be due to their role as co-factors in enzymatic processes. In particular, copper and zinc belong to the group of “antioxidant metals”, assisting the

function of antioxidant enzymes. Iron may be implicated through its role as a regulatory factor for angiogenesis. Distinct from these suggested mechanisms for uptake, the demand for increased blood supply for a growing tumour provides a basis for the accumulation of many other elements, including electrolytes such as potassium.

The present study is based on the detection of the X-ray fluorescence (XRF) emitted by the elements present in the tissue specimens when they are irradiated with photons of energy close to optimal. Measurements were performed at the European Synchrotron Radiation Facility (ESRF), working on the beamline BM28, operated as an EPSRC-funded facility under the acronym XMaS [1]. The objective of the experiment was the quantification of the levels of iron, copper, zinc and potassium in 80 breast tissue specimens.

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2. Materials and methods

2.1. Breast tissue samples

The tissue samples measured for this study were obtained from mastectomies, lumpectomies and breast reduction surgery. The tissue obtained from mastectomies or lumpectomies, was generally taken from the site of a tumour (invasive ductal carcinoma), although in a number of cases healthy tissue from areas distant to the tumour was also excised. Investigations were performed for 20 paired samples, 20 tumours that were unpaired with healthy tissue, and 20 samples from breast reduction procedures. Following excision, the samples were kept frozen at $-85\text{ }^{\circ}\text{C}$, no processing or sample preparation taking place between excision and measurement.

2.2. Experimental procedure for the XRF measurements

The high degree of linear polarization of the synchrotron beam was exploited in order to reduce the scatter signal received by the detector. A 90° geometry between synchrotron beam and detector was arranged leading to the reduction of the scatter signal. This arrangement leads to improved statistics for the fluorescence signal and reduced measuring times therefore increased throughput.

To provide for maximum production of fluorescence, the incident beam energy was tuned to 500 eV above the absorption edge of each of the elements, Fe, Cu and Zn. K concentrations are sufficiently large in tissue to have allowed its simultaneous measurement with Fe. The quantification of the elements was achieved through measurements made on calibrated solutions. Details of the experimental set-up and methodology can be found in Geraki et al. [2].

Quantification required estimation of the areas under the scattered and fluorescence photopeaks, the scattered photons signal being required for normalisation. This leads to requirement for estimating the relative scattering cross-sections for the two types of sample measured, namely water (the basis of the standard solutions) and tissue. For this, it was first necessary to determine the relative amounts of adipose and fibroglandular tissue,

the two main constituents of breast tissue, each having different scattering cross-sections. This was achieved using an energy-dispersive X-ray diffraction (EDXRD) system.

2.3. Experimental procedure for the EDXRD measurements

The momentum transfer values at which adipose and fibrous tissues most strongly diffract are 1.1 and 1.5 nm^{-1} , respectively, [3]. A tungsten target X-ray tube system was used at 70 kV_p and 15 mA , the scatter angle being set at 6° . A HPGc detector was used for collection of the scattered photons. The samples, mounted in 35 mm slide frames with windows of mylar, were placed on a translator to allow acquisition of diffraction spectra from three points on each specimen. The beam size on the samples was $1 \times 2\text{ mm}$. The resulting spectra for each sample were averaged prior to further analysis.

3. Analysis and results

3.1. Analysis of XRF and EDXRD spectra

XRF spectra from standard solutions were analysed using the software PeakFit (PeakFit™ SPSS Inc, AISN Software Inc.). The ratios of evaluated fluorescence to scattered photopeak areas were used to obtain a least squares fit between normalised element fluorescence and concentration. The spectra acquired from the specimens were also analysed with PeakFit and the photopeak areas were evaluated.

An example of the diffraction spectra collected can be seen in Fig. 1. The two spectra represent separately averaged results from the healthy and tumour tissue samples, showing the characteristic diffraction peaks of adipose and fibrous tissue and the relative intensities. The diffraction spectra were also analysed using PeakFit. The evaluated areas were corrected to account for the different cross-sections for coherent scattering from the two materials. The difference in cross-sections was calculated using the molecular form factors tabulated by Poletti et al. [3]. From the corrected areas the relative amounts of adipose and fibrous tissue in each specimen were evaluated.

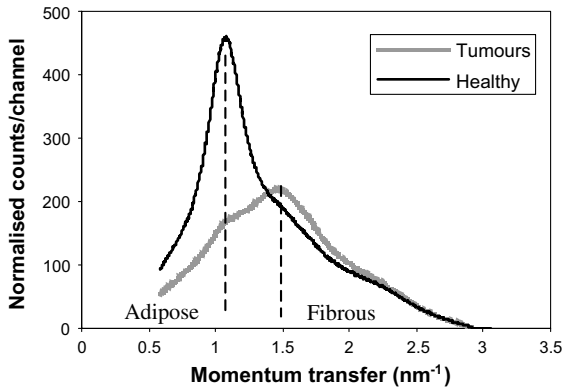


Fig. 1. Diffraction spectra acquired from healthy and cancerous breast tissue specimens.

3.2. Correction procedure

The photons scattered from the samples and standards comprise of photons suffering coherent and incoherent scattering, detector resolution being insufficient to allow their separation. It was therefore necessary to evaluate the relative amounts of coherent/incoherent photons, using Monte Carlo analysis (see Section 3.3). The total scatter peak area of tissue spectra, T , was corrected with respect to water (calibration solutions) to T' , according to the relative proportions of adipose and fibrous tissue, coherent and incoherent scattering, using the following equation:

$$T' = \left(\frac{ac + fc'}{ac_a + fc_f} + \frac{ai + fi'}{ai_a + fi_f} \right) T, \quad (1)$$

Table 1

Overview of statistical analysis of K concentration in tissue specimens

K	Paired samples		Independent samples	
	Healthy	Tumours	Healthy	Tumours
Range (ppm)	0–1000	0–2900	0–416	0–2561
Mean (ppm)	438	1112	210	1032
S.D. (ppm)	321	735	123	619
Median (ppm)	390	1049	264	1003
2.5–97.5th centile (ppm)	0–992	0–2534	0–400	0–2146
Mean tumour/mean healthy concentration		2.5		4.9
p values		0.001		7E–06

where a and f are relative amounts of adipose and fibrous tissue in specimen, c and i the relative amounts of coherently and incoherently scattered photons from adipose, c' and i' the relative amounts of coherently and incoherently scattered photons from fibrous, c_a and i_a the ratio of coherently scattered photons from adipose to coherently scattered photons from water and the equivalent ratio for incoherently scattered photons and c_f and i_f are the ratio of coherently scattered photons from fibrous to coherently scattered from water and the equivalent ratio for incoherently scattered photons.

3.3. Monte Carlo analysis

The Monte Carlo code used was EGS4 [4], including the low energy (1–10 keV) X-ray scattering extension LSCAT [5], and adapted for XRF

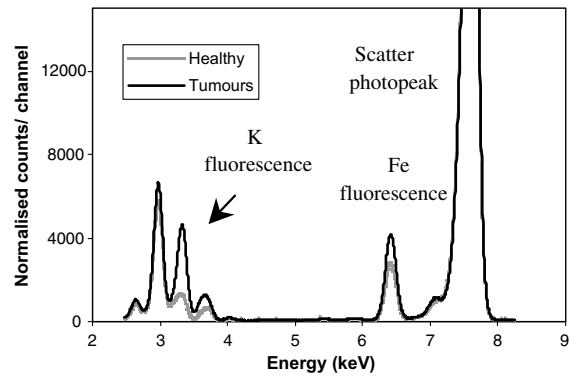


Fig. 2. Comparison between average healthy and average tumour XRF spectra obtained for iron and potassium.

geometry [6]. LSCAT also allows for the linear polarisation of the incident beam. The calculations led to estimation of the weighing factors (c , i , c' and i') and the correction parameters (c_a , i_a , c_f and i_f) for the three values of excitation beam energy.

3.4. Results

Fig. 2 shows an example of the comparison between the averaged XRF spectrum collected

from all of the normal tissue samples and the spectrum resulting from the averaging of all the tumour sample spectra, at this instance for potassium and iron.

The distributions of elemental content within the different groups of specimens are all positively skewed, being typical of biological media [7]. In evaluating differences between healthy and diseased tissue two comparisons were conducted. The first was between paired normal and tumour

Table 2
Overview of statistical analysis of Fe concentration in tissue specimens

Fe	Paired samples		Independent samples	
	Healthy	Tumours	Healthy	Tumours
Range (ppm)	0–43.7	0–58.4	0.6–24.2	0–58.4
Mean (ppm)	14.1	21.7	7.0	18.8
S.D. (ppm)	12.9	17.8	8.0	16.4
Median (ppm)	10.0	21.4	3.3	15.9
2.5–97.5th centile (ppm)	0–40.9	0–57.0	0.6–24.1	0–55.3
Mean tumour/mean healthy concentration		1.5		2.7
p values		0.16		0.002

Table 3
Overview of statistical analysis of Cu concentration in tissue specimens

Cu	Paired samples		Independent samples	
	Healthy	Tumours	Healthy	Tumours
Range (ppm)	0–1.27	0–2.25	0–0.60	0.08–2.25
Mean (ppm)	0.33	0.96	0.29	0.95
S.D. (ppm)	0.32	0.60	0.18	0.43
Median (ppm)	0.30	0.85	0.29	0.95
2.5–97.5th centile (ppm)	0–1.44	0.04–2.13	0–0.6	0.08–2.0
Mean tumour/mean healthy concentration		2.9		3.3
p values		0.002		4E–07

Table 4
Overview of statistical analysis of Zn concentration in tissue specimens

Zn	Paired samples		Independent samples	
	Healthy	Tumours	Healthy	Tumours
Range (ppm)	0–8.1	0–21.3	0.5–3.7	0.1–21.3
Mean (ppm)	2.9	6.9	1.8	7.7
S.D. (ppm)	2.0	5.8	1.0	7.2
Median (ppm)	2.3	5.2	1.7	7.2
2.5th–97.5th centile (ppm)	0.5–7.0	0.3–18.5	0.7–3.7	0.5–19.3
Mean tumour/mean healthy concentration		2.4		4.2
p values		0.02		3E–05

samples and the second between all tumour samples and the normal tissues obtained from healthy women. The summary statistics of Tables 1–4 describe elemental distributions in the three groups of tissue. The p values are the product of non-parametric comparison (equivalent to the t -test) between the groups representing the healthy and diseased tissue samples. For the paired samples, the Wilcoxon signed rank test was used, while the two groups of independent samples were compared using the Mann–Whitney test. p values lower than the cut-off point of 0.05 indicate that the two groups differ significantly with respect to the property that was used for the comparison [7].

4. Discussion

Results reveal increased concentrations of all elements in the tumours, summarised by the ratio of mean tumour to mean healthy concentrations and by the p values which further suggest significantly distinct groups of samples. The concentration elevation is least for Fe and most pronounced for K. Higher levels of Fe, Zn and K are observed in healthy tissues obtained from areas close to tumours compared to normal tissues obtained from healthy women. This is revealed by the significantly different mean values of element concentrations (35% deviation from average for K, 33% for Fe and 23% for Zn). The p values from non-parametric comparisons between the two groups of normal tissue also suggest the possibility of differentiation (0.03 for K, 0.06 for Fe and 0.07 for Zn). One interpretation for this apparent difference between the two types of healthy specimens is that the physiological processes leading to the accumulation of elements in the tumours – increased cellular and enzymatic activity – could have affected the composition of the healthy tissue in the margin surrounding lesions. Investigation of this is required, particularly since most breast cancer studies involving trace element content have been based on measurements of paired samples.

A feature distinguishing this study from previous investigations is the absence of sample preparation. Most studies have been performed on dried and occasionally homogenised samples, processes

that disturb the tissue from its natural physiological state. Ng et al. [8] have noted that the elevation of elemental content in tumours was significantly reduced when concentrations were adjusted using the wet-to-dry ratio of the samples. The same study reported that the wet-to-dry ratio varied significantly amongst specimens, not only of different type but also between samples of the same group. In the light of this, evaluated trace element concentrations from dried samples should be regarded as incomplete in the absence of wet-to-dry ratios for individual specimens. It would appear that study of fresh, unprocessed specimens is preferable.

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