J. Synchrotron Rad. (2000). **7**, 348–352

Breast cancer diagnosis using scattered X-rays

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(*Received 1 May 2000; accepted 18 July 2000*)

Small-angle X-ray diffraction data has been collected from 99 'core-cut' breast tissue specimens representing a number of different pathologies. Data in the range 75–1390 Å have been compared with controls from patients with no breast disease. Bessel functions and Bragg maxima resulting from the fibrillar structure of collagen have been identified. The Bragg maxima indexed onto a 649 Å lattice. Systematic differences in the intensities and *D*-spacings between the collagen of malignant, benign and normal tissue groups have been clearly demonstrated and quantified. These differences appear to be due to a significantly lower structural order within the malignant tissues. Possible explanations for this are discussed and the potential for utilizing this observation in cancer diagnosis is considered.

Keywords: breast cancer; SAXS; collagen; diagnosis; enzyme degradation.

1. Introduction

All disease states are associated with changes in cellular and/or tissue biochemistry with consequent effects on tissue structure and, thus, it is reasonable to suggest that the presence and type of disease may be detected by analysing tissue molecular structure. The ability of X-ray diffraction to provide such molecular information on live tissue has lead us to investigate the possibility of exploiting coherently scattered X-rays as a diagnostic tool. Of particular concern is the early detection and diagnosis of breast cancer.

The current screening method for breast cancer is X-ray mammography which can reduce population mortality (Nystrom, 1993). This is because mammography can identify a proportion of breast cancers while small and before they have spread. The overall sensitivity of screening mammography for breast cancer is around 90% (Mushlin *et al.*, 1998). The sensitivity is reduced in younger women, in those women with a dense background pattern and in women on hormone replacement therapy (Rosenberg *et al.*, 1998; Sibbering *et al.*, 1995). Mammography is non-specific and less than 20% of those women recalled for further assessment following a suspicious mammogram prove to have cancer (Bjurstam *et al.*, 1997). Many women recalled require percutaneous or surgical breast biopsy to confirm the nature of their breast lesion. This is invasive and subject to sampling error. Any technique capable of reducing the need for breast biopsy and/or aiding the analysis of biopsy specimens, especially in the presence of sampling error, would be highly advantageous.

Collagen is one of the major components of breast tissue and has been extensively studied by various techniques including small-angle X-ray scattering. Collagen exists in different functional types that have different primary structures. The most abundant in connective tissue are the fibre-forming collagens (*e.g.* types I, II, III, V, XI) which self assemble into cross-striated fibrils forming larger fibre structures which yield excellent X-ray diffraction data. Collagens are major components of the extracellular matrix (ECM), the degradation and penetration of which are known to be significant processes in the morbidity and mortality of cancer (Schonermark *et al.*, 1997). The exact role of the ECM in cancer is not well understood despite considerable research (Flug & Kopf-Maier, 1995; Lipponen *et al.*, 1994; Siegel & Malmsten, 1997). Nevertheless, it has been shown that its structure is seriously disturbed in malignant breast lesions (Raymond & Leong, 1991). The collagen associated with breast carcinoma has attracted some recent attention. Work by Schor *et al.* (1994) has suggested that normal tissue adjacent to a malignant tumour has functionally anomalous 'foetal like' fibroblasts. It has also been shown (Pucci-Minafra *et al.*, 1998) that the invasive tumour expansion of breast carcinomas is characterized by drastic changes in the collagen scaffold which are correlated with significant biochemical changes.

We have performed small-angle X-ray scattering measurements at the Synchrotron Radiation Source at Daresbury Laboratory in order to provide an initial

Table 1
Numbers and types of samples analysed.

Specimen type	Number of patients	Average age of patients	Number of samples	Type of samples
Reduction mammoplasty	18	38.5	29	Samples dissected from cosmetic breast reductions
Mastectomy	18	61.4	63	Core cuts of invasive breast carcinomas and tissue at various distances from tumour
Direct core biopsy	7	36.1	7	Core cuts of specific benign lesions
Total	43		99	

assessment of the collagen structures within various breast tissues (see Table 1).

2. Experimental

Core-cut biopsy specimens were obtained from patients who were being investigated following suspicious mammograms and who were shown by histology to have benign breast disease. A Bard Magnum instrument (14 gauge needle and 22 mm throw) was used to excise the specimens which were stored frozen at 243 K in buffered saline. Core cuts were also extracted from mastectomy samples of patients with breast cancer in which the lesions were palpable. These specimens were snap frozen in liquid nitrogen and, where possible, taken at varying distances from the lesion. Excised tissue was also obtained following cosmetic reduction mammoplasty from patients having no known breast disease. This was frozen at 243 K in buffered saline and samples similar to core cuts were subsequently dissected for diffraction analysis. In total, 99 specimens from 43 patients were examined (see Table 1). All samples were maintained in LN_2 or frozen and allowed to defrost naturally a few minutes prior to X-ray diffraction. Samples were inserted into 1.0 ± 0.25 mm-diameter special glass capillary tubes (nominal 10 μm wall thickness, provided by Pantak Ltd) with the aid of a vacuum system. This method partially aligned the collagen as the specimen was introduced into the tube. Physiological saline was inserted above and below each specimen to ensure that the specimen remained fully hydrated during the data collection.

Diffraction data were collected at station 2.1 of the Daresbury SRS (Towns-Andrews *et al.*, 1989) using a beam size of $\sim$0.5 mm $\times$ 0.5 mm at the sample and a wavelength of 1.54 Å. The sample-to-detector distance (SDD) was 6.52 m and rat-tail tendon was used as a calibrant. The scattering range was 75–1390 Å. Data were recorded using a 200 mm $\times$ 200 mm imaging multiwire proportional counter operated at 512 by 512 pixels (Lewis, 1994). The region between the specimen and detector was evacuated to minimize air scatter. Exposure times were 300 s, and typically resulted in 10^8 counts per image with the brightest pixels having $\sim 10^5$ counts per pixel and the weakest ones having $\sim$100 counts per pixel. The length of the specimens inside the capillary tubes varied from 3 mm to 20 mm. To average tissue inhomogeneities, images were recorded with the capillary oscillating vertically through the beam over the length of each specimen. For each specimen the capillary was also translated vertically to a position where no tissue was in the beam and an image was recorded of the blank capillary tube.

Diffraction data reduction was systematic. Following removal of detector artefacts, the background scattering from the specimen cell was subtracted. Corrections were applied for incident beam decay and the two-dimensional images were radially integrated. The subsequent one-dimensional data were multiplied by x^2 (x = SDD tan 2θ) to reduce the dynamic range and facilitate peak fitting. A fitted quadratic background derived from the data in the scattering vector (S) ranges 0.005–0.007 Å^{-1} and 0.008–0.009 Å^{-1} was removed before fitting Gaussian peaks. The number of peaks required to obtain satisfactory fits varied significantly from sample to sample with a maximum of 11 and a minimum of four. A high accuracy of fit was obtained to all data, with the lowest Pearson correlation coefficient being 0.997.

Following the diffraction measurements, the specimens were placed in 10% buffered formalin and transferred to the Department of Pathology at City Hospital, Nottingham, for histological examination. The specimens were routinely processed; paraffin wax-embedded and haematoxylin- and eosin-stained sections were examined by a consultant breast histopathologist (SEP). Cases were classified as normal, benign or invasive mammary carcinoma. No *in situ* carcinomas were included. The proportion of fibrous tissue in the core and the percentage area of tumour were determined semi-quantitatively. Histological grade, tumour type and size were also determined.

3. Results

Examples of two-dimensional scattering patterns are shown in Fig. 1. It was not the aim of this work to perform a full molecular structure analysis of breast tissue but rather to identify features in the X-ray scatter distributions which may act as markers of disease. However, it was helpful to recognize major features to aid parameterization of the data. The two major tissue components of the breast, *i.e.* collagen and adipose tissue, have well known and quite different scattering distributions. Within the range of angles studied here, the scattering from adipose tissue is relatively featureless. In contrast, two sets of intensity maxima from collagen were evident in all the data. One set arises as a

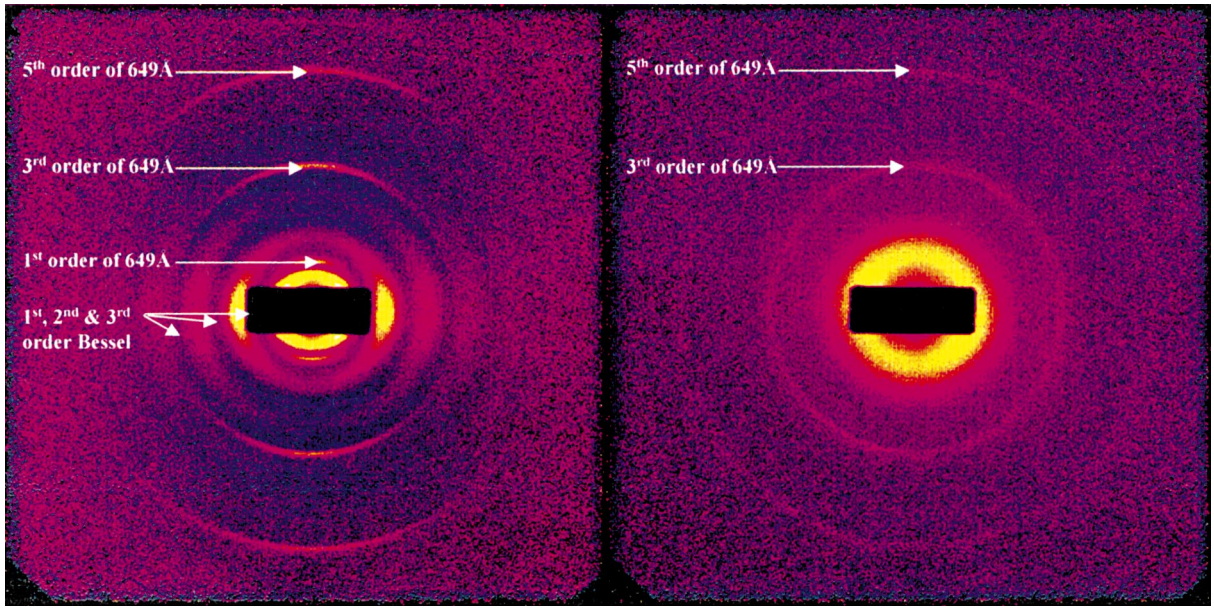

Figure 1
Diffraction images of two samples from the same breast. Left: apparently normal breast tissue. Right: tumour tissue.

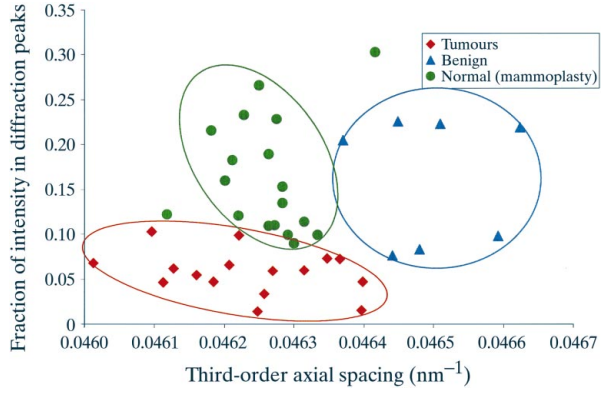

Figure 2
Scatter plot of the fraction of scattered intensity in the diffraction peaks *versus* the spacing of the third-order axial reflection. Ellipses are shown to illustrate the clustering.

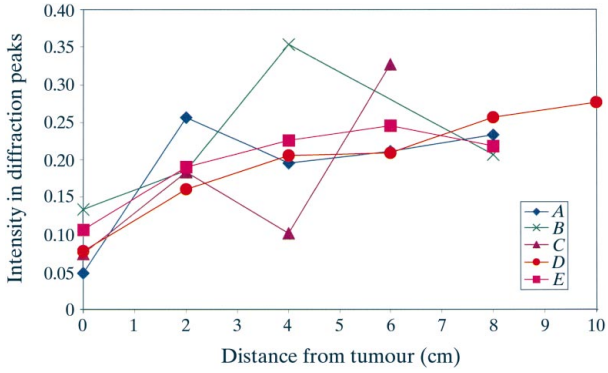

Figure 3
Variation of the fraction of intensity in the diffraction peaks with distance from the lesion for five patients having malignant breast disease.

consequence of the staggered arrangement of tropocollagen molecules within the fibrils and is the well known axial *D* spacing (Bigi & Roveri, 1991). In our data, these indexed onto a lattice with a fundamental spacing of approximately 649 Å (see Fig. 1). The third and fifth orders were most apparent but the first order and, less-frequently, the second order were also visible. The fourth order was visible in only one sample. The second set of peaks arises from the lateral packing of the fibrils in a quasi-hexagonal lattice (Eikenberry, Brodsky & Parry, 1982; Eikenberry, Brodsky, Craig & Parry, 1982). Following the treatment of Eikenberry *et al.*, the diffracted intensity can be approximated by a Bessel function. The lower-order peaks (0–3) were often visible as illustrated in Fig. 1.

It was immediately apparent from the one-dimensional data that the fraction of the total scattered intensity contained in the axial and Bessel peaks was much less in tumour tissue than in either normal tissue or benign lesions. Also, the spacing of the third axial peak was significantly greater in the seven benign lesions than for the other specimens. Fig. 2 shows these two parameters plotted against one another. Tumour samples cluster towards the bottom left, normal samples towards the top and benign lesions towards the middle right. The data from those samples of 'normal tissue' but from a breast containing a malignancy all fall in the region between normal and malignant tissue (not shown). The differences between the tissue types are highly statistically significant as shown by the P values resulting from a two-tailed t test assuming different variances. For tumour to benign, $P = 2.7 \times 10^{-5}$; for benign to normal, $P = 1.6 \times 10^{-4}$; and for normal to tumour, $P = 3.3 \times 10^{-7}$. Moreover, a linear regression between the fraction of intensity in the diffraction peaks

against the distance from the tumour is statistically significant ($P = 3.8 \times 10^{-4}$) (see Fig. 3).

The data have been carefully examined for other correlations, which may confound our observations. Further experiments showed that no significant differences in scattering patterns were observed when the different freezing techniques were utilized. We have also considered a previous observation (Ferguson *et al.*, 1992) that the ECM undergoes changes during the menstrual cycle. However, this does not appear to involve collagen types I, III, VI and VII and our data are predominantly sensitive to types I and III. In addition, roughly 60% of the patients were post menopausal and it seems unlikely that the menstrual cycle had a major effect on our data. The average age of the breast cancer patients was higher than those with benign lesions or those having cosmetic surgery (see Table 1). Since breast collagen content decreases with age, some of the lack of ordered diffraction observed in tumour samples could be correlated with patient age. However, a regression of all the data against age produced a Pearson correlation coefficient of only 0.3. Moreover, in all cases the fraction of scatter in the peaks was significantly greater for 'normal' tissue extracted from breasts containing a malignant tumour than from the tumours themselves (Fig. 4). These results strongly suggest that age is not the explanation of our data. Furthermore, although the lack of diffraction peak intensity could have been consistent with the tumour specimens containing less collagen, this was not supported by histopathological evidence, which showed an average of 70% fibrous tissue for the normal samples and 90% for the tumour samples. We therefore conclude that the fraction of X-rays scattered into discrete diffraction maxima is correlated with disease.

4. Discussion

The fraction of the scattered intensity contained in the Bragg peaks represents a sensitive measure of the supramolecular order within the collagen. Thus, X-ray diffraction appears to demonstrate a significant reduction in the collagen structural order of malignant tissues of the breast. This observation is supported by other work. For example, it is known that metalloproteinases responsible for collagen degradation are associated with the malignant phenotype in a variety of human tissues including the breast (Coussens & Werb, 1996). It has also been reported (Kauppila *et al.*, 1998) that breast tumours display aberrant collagen bundle organization in the tumour stroma. Furthermore, extensive alteration of the ECM has been observed in invasive colorectal carcinomas where the collagen derangement is attributed to both enzymatic degradation and altered neosynthesis. Our data are entirely consistent with these observations and suggest that the underlying collagen structure is disrupted at the molecular level in breast carcinomas.

The difference between the benign third axial spacings and those from other tissues is not understood. The difference (0.004626–0.004650 Å^{-1}) is six times less than the difference between normal human breast collagen and rat tail tendon (0.004626–0.004477 Å^{-1}). Since rat tail tendon is almost pure type I collagen whilst breast tissue contains types I and III, the change may suggest a difference in the ratio of collagen types between fibroadenomas and other tissues.

These diffraction experiments have indicated that high-quality interpretable diffraction data can be rapidly produced from breast core-cut biopsy specimens. We have demonstrated for the first time using diffraction a remarkable and systematic difference between the X-ray scattering from normal, benign and malignant breast tissue collagen. Our findings indicate that it may be possible to use molecular structure characteristics of breast tissue as novel markers of disease progression. Clearly further investigations are warranted to determine the tumour stage sensitivity of the technique and its ability to distinguish between tumour types. We are also currently considering the possibility of collecting such structural information *in vivo*.

We are grateful to the management and staff at Daresbury Laboratory for support and helpful advice.

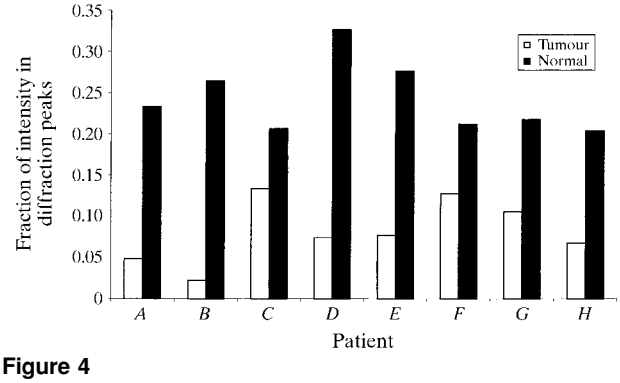

Figure 4
Comparison of scattered intensity in the diffraction peaks for tumour samples and 'normal' tissue taken from the same breast.

References

Bigi, A. & Roveri, N. (1991). *Fibre Diffraction: Collagen Handbook of Synchrotron Radiation*, edited by S. Ebashi, M. Koch & E. Rubenstein, Vol. 4, pp. 199–239. Amsterdam: Elsevier.

Bjurstam, N., Björneld, L., Duffy, S. W., Smith, T. C., Cahlin, E., Eriksson, O., Hafström, L. O., Lingaas, H., Mattsson, J., Persson, S., Rudenstam, C. M. & Säve Söderbergh, J. (1997). *Cancer*, **80**(11), 2091–2099.

Coussens, L. M. & Werb, Z. (1996). *Chem. Biol.* **3**, 895–904.

Eikenberry, E. F., Brodsky, B., Craig, A. S. & Parry, D. (1982). *Int. J. Biol. Macromol.* **4**, 393–398.

Eikenberry, E. F., Brodsky, B. & Parry, D. (1982). *Int. J. Biol. Macromol.* **4**, 322–328.

Ferguson, J. E., Schor, A. M., Howell, A. & Ferguson, M. (1992). *Cell Tissue Res.* **268**, 167–177.

Flug, M. & Kopf-Maier, P. (1995). *Acta Anat.* **152**, 69–84.
Kauppila, S., Stenbäck, F., Risteli, J., Jukkola, A. & Risteli, L. (1998). *J. Pathol.* **186**(3), 262–268.
Lewis, R. A. (1994). *J. Synchrotron. Rad.* **1**, 43–53.
Lipponen, P., Ji, H., Aaltomaa, S. & Syrjanen, K. (1994). *J. Cancer Res. Clin. Oncol. (HL5)*, **120**(11), 645–650.
Mushlin, A. I., Kouides, R. W. & Shapiro, D. E. (1998). *Am. J. Prev. Med*. **14**(2), 143–153.
Nystrom, L. (1993). *Lancet*, **341**, 973–978.
Pucci Minafra, I., Andriolo, M., Basiricò, L., Alessandro, R., Luparello, C., Buccellato, C., Garbelli, R. & Minafra, S. (1998). *Carcinogenesis*, **19**(4), 575–584.
Raymond, W. A. & Leong, A. S. Y. (1991). *Pathology*, **32**, 291–297.
Rosenberg, R. D., Hunt, W. C., Williamson, M. R., Gilliland, F. D., Wiest, P. W., Kelsey, C. A., Key, C. R. & Linver, M. N. (1998). *Radiology*, **209**, 511–518.
Schonermark, M. P., Bock, O., Buchner, A., Steinmeier, R., Benbow, U. & Lenarz, T. (1997). *Nature Med.* **3**(10), 1167–1171.
Schor, A., Rushton, G., Ferguson, J. E., Howell, A., Redford, J. & Schor, S. L. (1994). *Int. J. Cancer*, **59**, 25–32.
Sibbering, D., Burrell, H. C., Evans, A. J., Yeoman, L. J., Wilson, R. M. & Robertson, J. F. (1995). *Breast*, **4**, 127–129.
Siegel, G. & Malmsten, M. (1997). *Int. J. Microc-Clin. Exp.* **17**(5), 257–272.
Towns-Andrews, E., Berry, A., Bordas, J., Mant, G. R., Murray, P. K., Roberts, I., Sumner, I., Worgan, J. S. & Lewis, R. A. (1989). *Rev. Sci. Instrum.* **60**, 2346–2349.