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***In Vitro* laser-induced fluorescence studies of breast tumors
following low-dose injection of Photofrin**

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ABSTRACT

A study in order to investigate the uptake of porphyrins in neoplasias in the female breast tissue and to evaluate the potential of fluorescence diagnostics for tumor demarcation was performed. Six women with positive mammography and cytological biopsies took part in the study. The patients were given Photofrin intravenously in a low dose of 0.35 mg/kg b.w. 24 hours before the planned surgical procedure. Immediately after surgery the pathological specimen were investigated with fluorescence spectroscopy following 405 nm laser excitation. Afterwards the investigated tissue sample was taken for histological preparation. The spectroscopic results were correlated with the histopathological diagnoses. The study shows a good demarcation between invasive or *in situ* cancerous tissue and the surrounding breast tissue, based on fluorescence data.

1. INTRODUCTION

Breast cancer is the most common cancer type attacking the female population of the Western world today¹. It is widely agreed that early detection is of great importance for improving the prognosis. As a result of this, screening programs have been initiated in many countries with mammography of women in certain age and risk groups and stimulation of self examination in the whole of the female population. Fine-needle aspiration is frequently used for final cytological diagnosis.

In some cases of lobular carcinoma without retraction phenomena and in certain types of invasive tumors with comedo structures, the X-ray mammography may give false negative results. Also the fine-needle aspiration procedure may fail to hit the diseased tissue and then also results in false-negative outcome.

The treatment procedures have always been under discussion trying to reach a compromise between a radical removal of the disease and a cosmetically satisfying result, due to the psychological impact this tumor has on the patient caused by its location, and its sometimes diffuse growth pattern. As in all oncological surgery it is crucial to locate the tumor margins during the surgical procedure. Also since the maintenance of the breast often is of cosmetically importance, it is desirable to remove as little tissue as possible.

Laser induced fluorescence has been used in patients for diagnosis of cancer in various locations, the lung⁶, bladder⁷ etc. and has shown promising results as a means of diagnosing tissue with malignant potential⁶. Laser-induced fluorescence might provide a means for improved management of breast cancer. We have performed a preliminary study on the endogenous fluorescence and the uptake and selectivity of the photosensitizer Photofrin® injected in low-dose in six women with malignant breast tumors. This was performed to examine the possibility of using laser-induced fluorescence for diagnostics of breast tumors, and the potential for using Photofrin® as a sensitizer for photodynamic therapy (PDT) in these tumors.

2. MATERIALS AND METHODS

2.1. Patients

The patients taking part in the study were women with cancer of the breast that was confirmed both through positive radiology and positive cytology. They were scheduled for surgical removal of the tumor. Willingness to participate in the study was declared after both oral and written information. Six patients were included in this preliminary study.

2.2. Photosensitizer

The photosensitizer used was Photofrin® (Quadra Logic Technologies, Canada) a hematoporphyrin derivative. The drug was administered approximately 24 hours before the planned operation, which was either a segmental resection or a mastectomy. The dose given was 0.35 mg/kg b.w. dissolved in 5% glucose and administered intravenously. No side effects were seen in conjunction with the injections. There were neither any reports of sensitization of the skin.

2.3. Laser-Induced Fluorescence

Immediately after operation the tumor was cut and one slice was taken for spectroscopic examination. For recording the tissue fluorescence we used a 600 μm single quartz fibre for transmitting the excitation light and collecting induced fluorescence light. The fibre tip was held vertically in contact with the tissue. A Laser Science Model VSL 337 pulsed nitrogen laser operating at 337 nm was used to pump a Laser Science dye laser, tuned to 405 nm. The fluorescence emission was conducted to the entrance slit of a Jobin Yvon 0.25 m grating monochromator equipped with an EG&G Model 1421 gated and intensified linear array detector connected to an EG&G OMA (Optical Multichannel Analyzer) mainframe.

Fluorescence spectra were obtained along a line covering the full length of the cut tumor surface and surrounding tissue. One spectrum was taken for every 1-2 mm along this line. To be able to orient the tissue after the histopathologic preparation, certain locations in the sample were marked.

2.4 Histopathology

After the spectroscopic examination had been performed the tissue slice was placed in formalin and a full-size section with Hematoxylin-Eosin staining was prepared. The section was taken from the side of the slice where the spectroscopic measurements were performed. In this way the points recognised on the histopathological section could be compared to the spectroscopically measured points. Going through the sections and recognising the points measured, one could well correlate the histopathologic diagnoses with the fluorescence spectra.

3. RESULTS

3.1 Laser Induced Fluorescence and Correlation with Histopathologic Diagnoses

Of the tumors included in the study the histopathologic diagnoses ranged from *in situ* changes to poorly differentiated intraductal carcinomas according to WHO's (World Health Organisation) classification.

Because of shrinking and bending of the specimen during histologic preparation, it was in a few cases difficult to decide the exact position for the recorded spectra. However, representative spectra for cancerous versus surrounding tissue were obtained for each specimen.

The carcinomas of the breast tissue often grow with a zone of connective tissue surrounding them. This can cause a quite varied histologic picture. Spectra were recorded from both a dense collagen-rich stroma infiltrated with tumor streaks and with normal ducts, from pure infiltrating tumor, from tumor embedded in a loose stroma with much fat and from pure adipose tissue, which is a common component in the breasts.

Following 405 nm excitation, representative fluorescence spectra in the region 450-750 nm were recorded for evaluation. The tissue autofluorescence from native chromophores gives rise to a broad intensity distribution in the lower half of the spectral window employed. The autofluorescence was evaluated as the signal intensity at 490 nm. The characteristic two peaks at 630 nm and 690 nm, representing the accumulation of photosensitizer, were used as a measurement of photosensitizer concentration. The intensity at 630 nm was evaluated. For each specimen a large number of spectra were collected. Data for the six resected specimen are collected in table I.

Samples	I (630 nm)			I (490 nm)			I (630 nm)/I (490 nm)		
	Tumor	Normal glands etc.	Pure fat	Tumor	Normal glands etc.	Pure fat	Tumor	Normal glands etc.	Pure fat
Samples # 1-4: Including poorly differentiated ductal carcinoma	0.25±0.22	0.14±0.18	0.12±0.11	3.63±2.10	6.13±5.11	0.52±0.34	0.15±0.12	0.05±0.04	0.22±0.08
Sample # 5: Including ductal carcinoma <i>in situ</i> , with transition to early invasive cancer	0.27±0.03	0.19±0.08	0.05±0.04	0.27±0.15	1.74 ±0.63	0.21 ±0.12	1.31 ±0.64	0.12±0.05	0.28±0.15
Sample # 6: Including ductal carcinoma <i>in situ</i>	0.24±0.16	0.09±0.02	Absent	1.36±0.57	2,72±1.16	Absent	0.20±0.15	0.04±0.01	Absent

Table I.

As we can see from the table, the intensity at 630 nm ranges from 1.8 to 2.7 times higher in the tumor tissue than in the multi-componental surrounding tissue. Comparing tumor tissue and the pure adipose tissue, the ratios for the ductal carcinomas and the *in situ* changes are 2.1 and 5.4, respectively.

As a mean the autofluorescence is always higher in the tumor surrounding tissue than in the tumor and it is very low in the pure adipose tissue. Taking the two together and looking at the ratio between the intensity at 630 nm and 490 nm it is ranging from 3 to 11 times higher in the tumor than in the normal tissue.

4. DISCUSSION

The aim of this study was to study laser-induced fluorescence for breast cancer diagnosis and to determine the degree of uptake of Photofrin in neoplastic tissue of the breast and its distribution between tumor and healthy tissue. The results are important for evaluating the potential of using Photofrin as a photosensitizing drug in diagnostic and treatment procedures of breast cancer. The dose given was low enough to avoid skin sensitization and therefore the signals from the hematoporphyrin derivative were rather low in intensity.

The selectivity in drug uptake was in the order 2:1 towards the surrounding breast tissue. Taking into account that the autofluorescence changes from normal to tumor tissue^{7,10}, the diagnostic value could be enhanced by adding this information. This can be done by forming the ratio of the intensities at 630 nm and 490 nm. This ratio is, in our case, higher in the cancerous than in the surrounding tissue. In the adipose tissue it is also quite high because of low autofluorescence in this type of tissue. However, the variation in growth pattern for the different tumor types from the necrotic comedo structures to the dense, collagen rich connective tissue, makes the evaluation of the spectroscopic information a little complicated. Since streaks of dense collagen tissue in the tumor can cause a suddenly high value at 490 nm compared to the signal from partly necrotic structures, the standard deviation in the autofluorescence is high. However, taking all information into account, the signal from the hematoporphyrin derivative, the autofluorescence and ratio between these two intensities it should be possible to predict the cancerous state of the tissue measured. However, it should be mentioned that we have not yet injected patients with benign tumors of the mammary glands so we cannot so far say anything about differentiating between malignant and benign neoplasias.

Based on this preliminary study we feel that it should be possible to use the hematoporphyrin derivative (Photofrin®) for diagnostic procedures in the breast tissue *in vivo*. The procedure could be applied during the fine-needle aspiration procedure, inserting the optical fibre through the needle and measuring the fluorescence before aspirating the cell sample. The fluorescence signals recorded in real time could be used to guide the biopsy sampling and enhance the value of the information available from cytology. The fluorescence technique could also be applied peroperatively to identify the margins of the tumor. In such a case it might be more suitable to use an imaging system¹¹ than the point monitoring device. Administrating treatment doses of hematoporphyrin derivative it should also be possible to perform

interstitial Photodynamic Therapy (PDT) preoperatively to reduce the tumor mass or to apply superficial irradiation of the tumor bed for elimination of remaining tumor cells.

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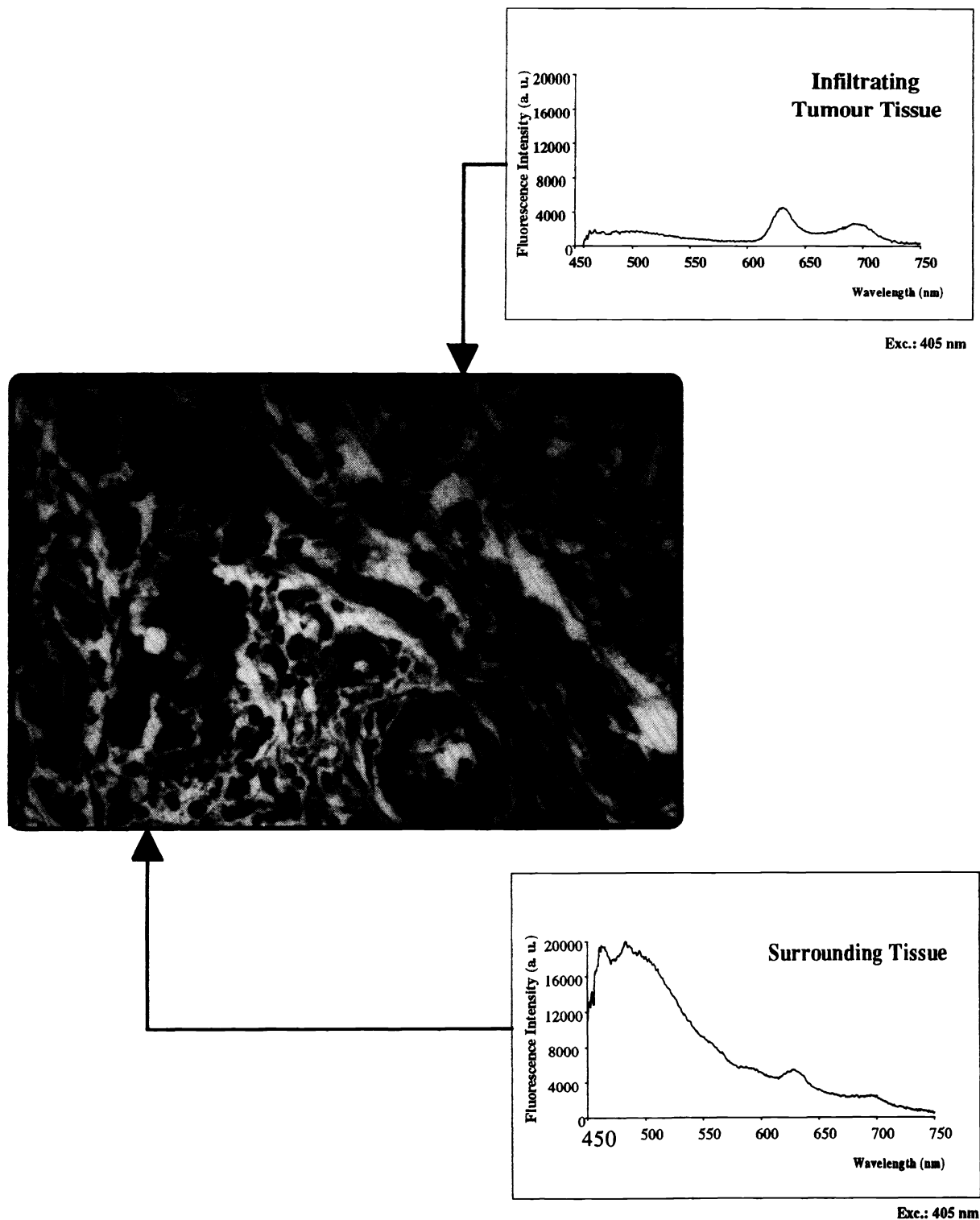


Figure 1. Laser-induced fluorescence spectra and histopathology from an invasive ductal breast cancer in a low-dose (0.35 mg/kg b.w.) Photofrin® injected patient.