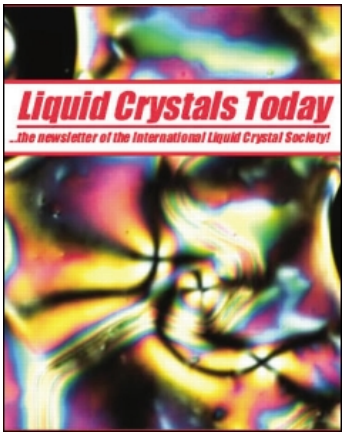

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The application of nematic liquid crystals for objective microscopic diagnosis of cancer

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Nematic liquid crystals (NLC) that were traditionally applied for detection of surface inhomogeneities in non-biological objects are proposed as an instrument for the objective microscopic diagnosis of malignancies in oncological practice. The specific alignment of NLCs on different types of tissue is exploited, a previously unknown phenomenon. Malignant tissue decorated with NLC due induces a homeotropic alignment while on benign tissues nematic materials are oriented close to planar mode. The relationship between this phenomenon and the different surface tension of malignant and nonmalignant tissues is revealed and the physical nature of these new phenomena is discussed.

1. Introduction

The application of thin layers of nematic liquid crystals (NLCs) to surfaces is used in science and technology for the detection of structural inhomogeneities on the surfaces of various materials and minerals. This non-destructive, highly sensitive technique allows the visualization of otherwise invisible peculiarities of the surface examined. Until recently, applications that use the properties of NLCs to decorate and therefore image biological or medically important samples were unknown, so the authors experiment demonstrating visualization of human skin epithelium stratification with NLCs [1] was particularly promising. It provided the impetus to evaluate the possibility of applying the nematic visualization method in cancer research.

The diagnosis of cancer is at least as important a problem as patient cure, a fact not widely appreciated. Traditionally, the microscopic diagnosis of cancer is based on the optical examination by the pathologist of a microtome tissue section stained with haematoxilin and eosin or with two other organic dyes and mounted on the object glass under fir balsam and the cover glass. The diagnosis of malignancy is not very difficult in most cases and is ruled on the basis of cellular atypism. The complexity of atypical features in cells makes such a diagnosis subjective, because not all cytological features are equally clear and in some cases the histological conclusion is based more on the pathologists experience and intuition than on true knowledge. In this context, a search for new objective method of cancer detection is extremely timely.

2. Experiments

The experimental setup used in our experiments involved a polarized light microscope, a microscope stage (that can be cooled or heated), and a video camera that allowed large screen image display and recording.

Twin sections of frozen human or dog tissues of thickness 4–5 νm were made with microtome. One sample was stained with haematoxilin and eosin and the pathologist using a routine method identified both malignant and non-malignant areas. The other twin section was examined via decoration with a nematic liquid crystal. The liquid crystal was applied to the sample in an isotropic state, achieved by mixing with an organic solvent. After evaporation of the solvent, the orientation of NLC molecules was defined only by the surface topography. After less than 1 minute the section was ready for examination. The image recorded could be reproduced after repeatedly washing out NLC with solvent and reapplying it many times over. The thickness of NLC layer was less than 1 νm.

More than 100 examples of human and canine malignant tumors, benign tumors and normal tissues were examined. The malignant tumors studied fell into four of the six existing histogenetic classes: epithelial neoplasms e.g., carcinomas, tumors of mesenchymal origin tissues e.g., sarcomas, lymphomas (originating from hemopoietic tissue) and melanomas (from melanin-containing tissue).

Decoration with the NLC clearly produced different orientation on the malignant and non-malignant tissues, and it was deemed necessary to understand the role of surface tension in causing this phenomenon. Consequently,

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further experiments were undertaken. Examining the wetting contact angles between the investigated surfaces and reference liquids allowed measurement of the surface tension of the tissue samples. A photographic method allowed a drops profile to be recorded, using back illumination to produce a shadow image.

Ten dry, frozen sections of tissue were examined. Each was 5–7 microns thick, fixed in 10% formalin solution and mounted on a slide. Some sections contained boundary areas of malignant and non-malignant tissues, which could be observed simultaneously. Two consecutive sections were produced for each sample examined, one of which was stained and examined by a pathologist in the tradiational way and the other of which was used in the surface tension measurements. Drops of water or the nematic liquid crystal MBBA (without a solvent) 1–2 mm in diameter were placed on the surfaces of the tissue specimens with a fine syringe. The samples were placed in a clear covered container (preventing water evaporation) and the drops profiles were photographed. The illumination system was equipped with an infra-red filter to prevent heating. The drops flattened gradually with time so profiles were recorded after 0.5, 2, 5 and 10 minutes. The contact angle between the drop profile and the surface was deduced from the images recorded.

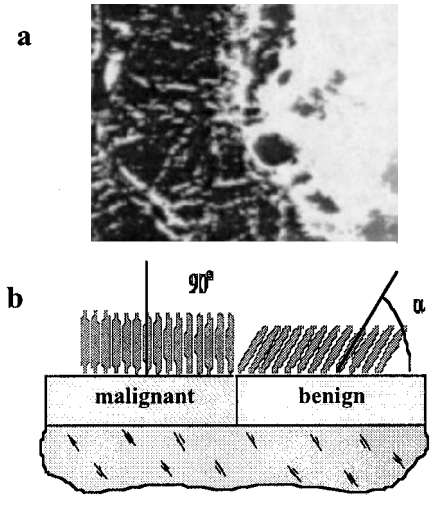

Figure 1. (*a*) Photo of rectal cancer (adenocarcinoma) malignant (left) and benign (right) tissues decorated by a NLC layer. (*b*) The model of NLC orientation on the tissue which depends on the surface tension value of NLC and tissue.

3. Results

Typical images of tissue samples decorated with thin layers of nematic liquid crystal are shown in figures 1 and 2. In every specimen examined, the areas of malignant tissue appeared black and areas of normal or benign neoplastic tissues were white or pseudocolored with different bright colors. Stroma (vessels and elements of the tumor skeleton) of malignant tumors were decorated as normal tissue (stroma of malignancies are benign in character). The display pattern was the same independent of the malignant tumor histogenetic type. If there were foci of necrosis in the malignant tumor, these were decorated as benign tissue. The interface between malignant and nonmalignant tissue was sharp without any intermediate state. These observations allowed us to formulate a new biophysical criterion for the *objective* diagnosis of malignant tumors.

The orientation of NLC molecules on a substrate can be considered in terms of the surface energy. The different orientation of NLC molecules on the various tissues investigated implies a difference in anchoring energy of NLC molecules on the surface of malignant and not malignant tumors. The interaction energy, F^S, of a nematic liquid crystal with a tissue surface is given by:

$$F^S = F^S_{IS} + F^S_{LC} \tag{1}$$

where F^S_{IS} is the isotropic part of the surface energy and F^S_{LC} is the anisotropic part. The analytic approximation for the surface energy of a nematic liquid crystal, F^S_a is given by the Rapini formula:

$$F^S_a = F^S_{IS} + F^S_a(\theta) + F^S_a(\phi) = F^S_{IS} + \frac{1}{2} W\theta \sin^2(\theta^S - \theta^{S0}) + \frac{1}{2} W\phi \sin^2(\theta\phi - \theta\phi^0). \tag{2}$$

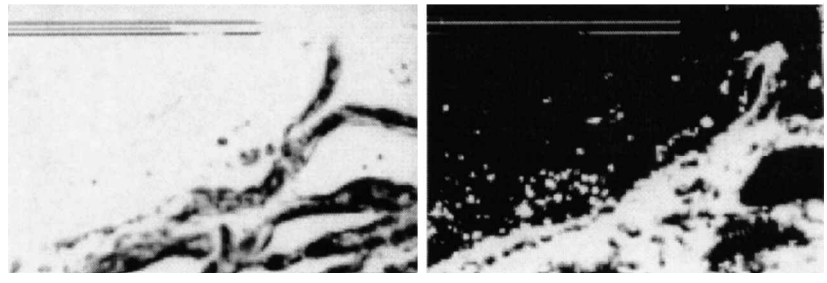

Figure 2. Leyomyosarcoma of the breast. Left: native tissue sections without any straining. Right: tissues decorated with NLC. The malignant tissue covered with NLC appears black. Magnification 100 ×.

Here $F_a^S(\theta)$ and $F_a^S(\phi)$ correspond to the polar and azimuthal energy components respectively. Usually $F_a^S(\theta) \gg F_a^S(\phi)$. Numerous experiments on nematic materials in a homeotropic orientation give surface anchoring energies in the range $W_h^S(\theta) = 10^{-3} \ldots 10^{-2}$ erg/cm^2, smaller than that associated with the planar orientation where $W_p^S(\theta) = 10^{-2} \ldots 1$ erg/cm^2. This means that the anchoring energy of a malignant growth is at least in two orders of magnitude less than that of healthy tissue. It explains the fundamental nature of the new criterion for cancer detection based on the orientation of nematic liquid crystals.

In order to confirm the validity of the above suggestion, it is important to confirm the distinct surface characteristics of different types of tissue using independent measurements. Determining the surface tension of malignant and non-malignant tissues should, in principle, allow this. It is well known that several biophysical and biochemical characteristics of malignant tumors differ from healthy tissue, though no information on surface tension appears in the literature in this context. It seems that this property of tissue has not previously been studied and it is both convenient and relevant to the phenomena discussed here.

The comparative surface tension experiments that were carried out on the ten tissue samples showed that in all cases the wetting contact angles on healthy tissues are greater than the contact angles on malignant tumors. On average, the difference between the two was as much as 30% of the absolute value of surface tension. In order to calculate the surface tension γ_{SV} of the malignant and healthy tissues, the Young and Neuman equation were used:

$$\gamma_{SL} = 2(\gamma_{LV} \times \gamma_{SV})^{1/2} \, e^{-0{,}000\,15(\gamma_{LV} - \gamma_{SV})^2}, \qquad (3)$$

where the subscripts S, L and V correspond to indices of a solid, liquid or vapour surface respectively and:

$$\gamma_{SL} = \gamma_{SV} - \gamma_{LV} \cos\theta. \qquad (4)$$

From our experiments and calculations, γ_L for MBBA at room temperature is 30 ± 1 dyn/cm^2. Further, for the tissues, $\gamma_{SV} = 26 \pm 1$ dyn/cm^2 for malignant tissues and $\gamma_{SV} = 32 \pm 1$ dyn/cm^2 for healthy tissues. The results of these experiments and calculations are in good agreement with the Kahn rule [2]: γ of malignant tissue (homeotropic orientation) $< \gamma$ of MBBA $< \gamma$ of healthy tissue (planar orientation).

4. Conclusion

A new phenomenon has been reported in which NLCs decorate malignant and non-malignant histological tissue samples differently, allowing their discrimination. Polarized light transmission microscopy used to view a thin nematic liquid crystal layer deposited on the tissue surface is a cheap and expressive method of providing extra information to a pathologist, aiding the diagnosis of tumors in difficult cases. Further investigation will help to determine the limitations and best areas of use for LC-vision in oncomorphology. It is believed that the phenomenon is connected with differing surface tension on different types of tissue. Not only does this provide the mechanism for the visualization technique, but possibly also allows a deeper understanding of the intrinsic reasons for the aggressive behaviour of cancer.

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