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Appearance of liquid crystals in acupuncture points

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Liquid crystals of biomolecules occur ubiquitously in biological systems, and to study the physiological mechanism of acupuncture, methods involving the detection of liquid crystals have been introduced. It is revealed that liquid crystals appear in the acupuncture point Zusanli 15 min after the appropriate stimulus in the acupuncture point Jiexi, which is located on the same meridian with Zusanli according to the Meridian Theory of Chinese Medicine, whereas liquid crystals are rarely observed in the points beside the Zusanli in the same condition. Subsequent studies on the composition of the liquid crystals detected, using energy dispersive X-ray analysis and histochemical analysis, suggest that the liquid crystals consist of proteins and perhaps phospholipids. The results show that these biomolecules, which are accumulated in the extracellular fluid of the acupuncture point, may function in the acupuncture effects, and moreover that liquid crystal detection can serve as a simple method to start studies of such complicated physiological phenomena.

1. Introduction

Biological structures, such as biomembranes, are the classical systems associating liquid crystals with living systems [1]. However, liquid crystals also accummulate in extracellular fluid during physiological or pathological processes. Up to now, only a few research projects of this kind have been done, e.g. during the processes of artery hardening, gallstone formation [1] and embryo development [2, 3].

Biomolecules existing in the extracellular fluid originate intracellularly. In some physiological or pathological situations, they assemble and form macroparticles which can be recognized as liquid crystal material. Thus, liquid crystal detection can serve as a method to judge a significant increase of these biomolecules or a conditional change causing their assembly, and consequently to promote the study of phenomena related to these biomolecules.

Acupuncture therapy originated in China about 2000 years ago [4]. It has become popular in the West since the 1970s, when President Nixon visited China [5, 6]. After a careful review (1997) of the researches on acupuncture, the US National Institutes of Health (NIH) admitted its efficacy at least in some situations [7]. According to the Meridian Theory of Chinese Medicine, on which traditional acupuncture is based, acupuncture is effective in many disorders [8, 9].

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Efforts to illustrate the mechanism of acupuncture's effects have been mainly based on neurology [10, 11]. The complicated characteristics of major physiological processes might also be shared by this phenomenon, and progress is slow. To seek a breakthrough in the area, we have introduced the method of detecting liquid crystals into the study.

In this paper, we report on the liquid crystal material found in an acupuncture point after the appropriate stimulus. The analysis of the liquid crystal material is described using polarized optical microscopy, electron microscopy, energy dispersive X-ray (EDX) spectroscopy and histochemical chromoscopy.

2. Experimental

2.1. *Animals and materials*

Fourteen adult Japanese white rabbits (body weight 2.5 ± 0.3 kg) were used in this study. 2,4-Dinitrofluorobenzene (DNBF) of biological reagent grade and 1-amino-8-naphthol-3,6-disulphonic acid monosodium salt (H acid monosodium salt) of pure chemical grade were purchased from the Third Reagent Factory of Shanghai (Shanghai, China). All other reagents and solvents were of analytical reagent grade.

2.2. *Equipment*

The liquid crystals were detected by microscopy employing a special polarizing optical microscope (XPl, The Optical Instrument Factory of Guizhou, China)

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equipped with a heating stage. The electron microscopic analysis and EDX analysis of the liquid crystals were made on a scanning electron microscope (SEM X-650, Hitachi, Japan) in conjunction with an EDX spectrometer (EDAX9100, Philips, the Netherlands).

2.3. *Procedures*

2.3.1. *Extraction of samples and polarizing optical microscopy analysis*

The experimental rabbit was placed on a wooden platform. A thin acupuncture needle was inserted into acupuncture point Jiexi (S41) for about 0.5 cm, and subsequently manipulated for 5–7 min following the traditional Chinese acupuncture technique (generally described as directing the needle up and down and rotating the needle clockwise and anticlockwise, repeatedly). The samples of tissue fluid were removed with a 0.5 mm syringe needle from the acupuncture point Zusanli (S36) at time intervals 0, 5, 10, 15, 20 and 30 min after the stimulus. Samples of two kinds served as controls, one from the Zusanli point before the stimulus in the Jiexi, the other from a point 1 cm to the right or 1 cm to the left of the Zusanli point after the stimulus in the Jiexi. The samples of tissue fluid were placed on glass slides for further analysis. They were first observed in the polarizing microscope, and the transition temperatures for the liquid crystals measured with the aid of the attached heating stage.

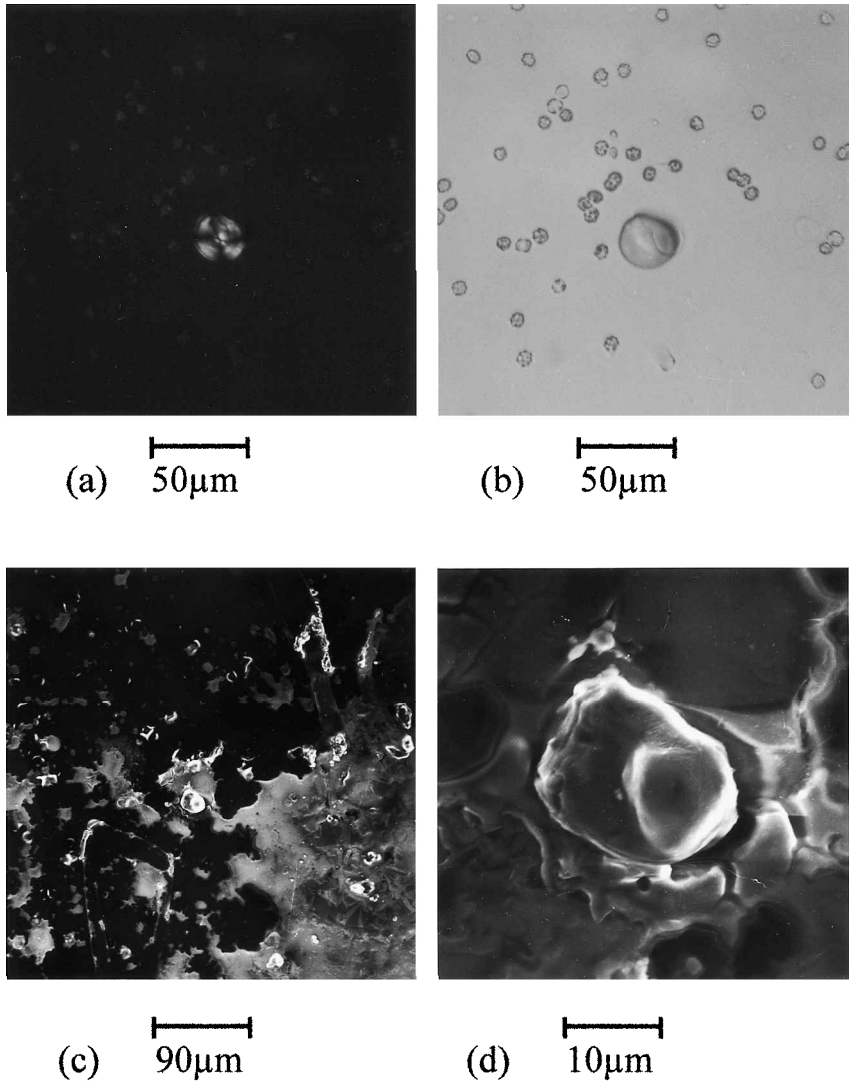


Figure 1. The morphology of a liquid crystal droplet obtained from the Zusanli 15 min after needle manipulation in the Jiexi, photographed (*a*) between crossed polarizers; (*b*) in unpolarized light, (*c*) and (*d*) in SEM. The small spheres in the background of (*b*) are blood cells, coming from capillary vessels injured when the sample was extracted.

2.3.2. *Electron microscopic analysis and EDX analysis*

The glass slides with the liquid crystal material were cut small enough to fit in the SEM. After drying at room temperature for 48 h, they were coated with a carbon film in the vacuum evaporator, and then used in the SEM, in conjunction with the EDX spectrometer, for analysis. The SEM images were obtained at 10.0 kV, while the EDX spectra were obtained at 25.0 kV.

2.3.3. *Histochemical analysis*

The samples containing the liquid crystal material were analysed using DNFB staining, a classical histochemical method for detecting general protein. The details of the method are to be found in major histochemistry manuals.

3. Results and discussion

3.1. *Appearance of the liquid crystals in the acupuncture points*

Liquid crystal material appeared in the tissue fluid samples extracted from the Zusanli 15, 20 and 30 min after the stimulus in the Jiexi. In the samples of 0, 5 and 10 min, liquid crystals seldom appeared. No liquid crystals appeared in the samples extracted from the Zusanli before the stimulus in the Jiexi. The samples extracted from the points beside the Zusanli rarely showed liquid crystals. Observed through crossed polarizers, the liquid crystal material formed droplets with a maltese cross, figure 1 (*a*), or some other birefringent form, figure 2 (*a*). Adding a cover glass over the material and pressing results in a transfiguration of the material;

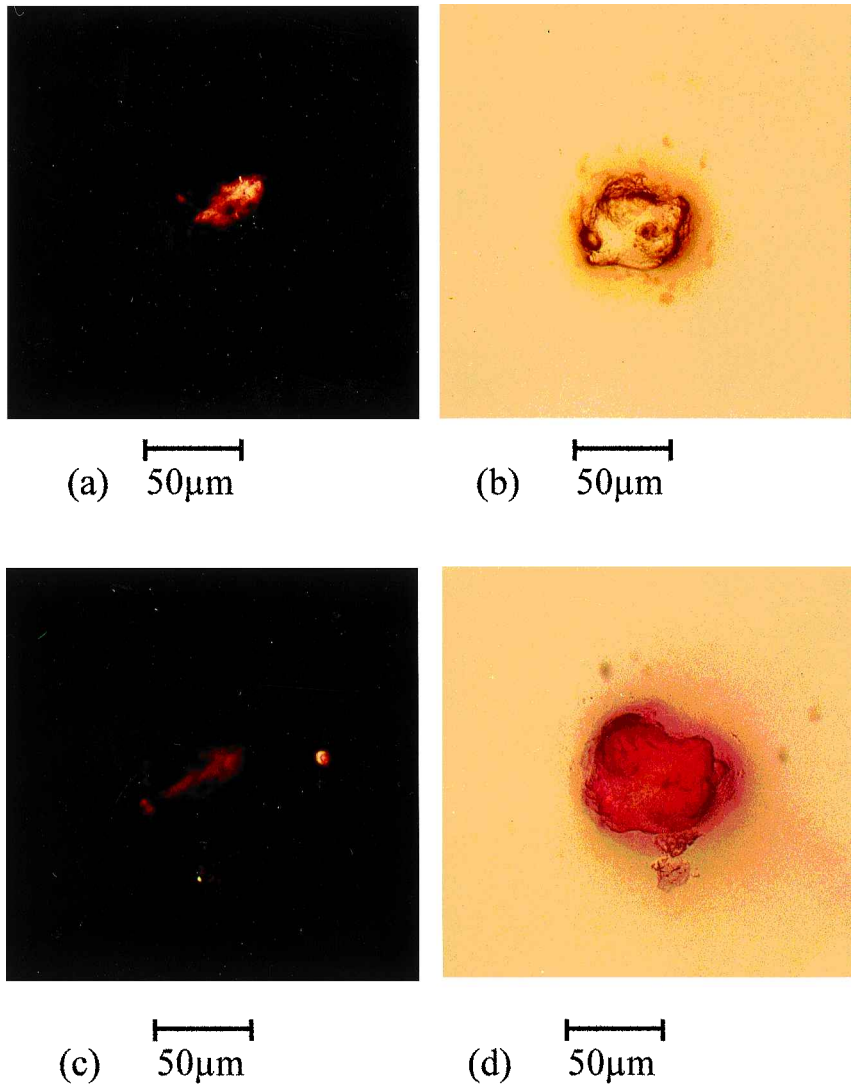
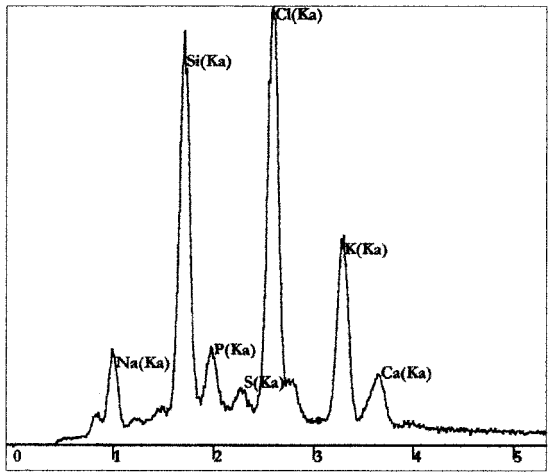


Figure 2. The DNFB chromoscopy of a liquid crystal sample obtained from the Zusanli 20 min after needle manipulation in the Jiexi. The sample was photographed before staining, (*a*) between crossed polarizers and (*b*) in unpolarized light; and after staining, (*c*) between crossed polarizers and (*d*) in unpolarized light.

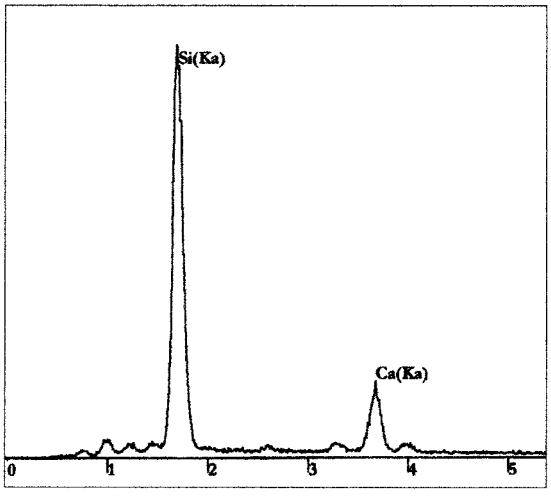
withdrawing the pressure reverses the change, which distinguishes the material from crystals. The liquid crystals transform to the isotropic state when the temperature rises to about 42°C.

3.2. *The composition of the liquid crystals*

Figure 1 shows the morphology of a sample of detected liquid crystal material, whose EDX analysis is shown in figure 3 (*a*). Figure 3 (*b*) is the analysis of an area beside the sample, showing high peaks for Si and Ca which were mainly contributed by the glass slide. Figure 3 (*a*) is the analysis of the area on the sample, showing peaks for Na, P, S, Cl and K, besides Si and Ca. The elements Na, Cl, K and perhaps some Ca may represent the inorganic ingredients included in the sample. The elements P and S mainly suggest the organic ingredients, in which P is associated with phospholipid or nucleic acid and S with protein or sulphated mucosubstances. Considering the sample's liquid crystallinity and morphotype, phospholipid and protein are the most likely ones to be in its composition [1].



(a) Weight%(normalized): Na, 16.65; Si, 22.90; P, 8.20; S, 4.63; Cl, 27.76; K, 14.69; Ca, 5.17.



(b) Weight%(normalized): Si, 80.75; Ca, 19.25.

Figure 3. The EDX spectra of the area (*a*) *on* and (*b*) *beside* the liquid crystal sample whose morphology is shown in figure 1. The two areas which were subjected to the EDX analysis are of the same scale, about $10 \times 10\,\mu m^2$.

DNFB staining, a histochemical method, though not used in a histological section, benefits our staining requirements for the liquid crystal sample by its *in situ* character. In figure 2, the particle is seen stained red, the typical colour for protein, a result strongly suggesting that the liquid crystal sample obtained from the acupuncture point contains protein as one of its major components. In this context, we recognize and emphasize that histochemical methods could play a role in research on the composition of biological liquid crystals.

3.3. *About acupuncture*

In the West, acupuncture is becoming an important form of alternative therapy. Debates about it lasted from the early 1970s to the end of the 1990s [12–15]. Reports from the NIH Consensus Panel on Acupuncture in November 1997 said that there are '*promising results, for example, showing efficacy of acupuncture in adult post-operative and chemotherapy nausea and vomiting, and in post-operative dental pain*' and there are '*other situations, such as addiction, stroke rehabilitation, headache, menstrual cramps, tennis elbow, fibromyalgia, myofascial pain, osteoarthritis, low back pain, carpal tunnel syndrome, and asthma*', in which '*acupuncture may be useful as an adjunct treatment or an acceptable alternative or be included in a comprehensive management program.*' [7].

In China, acupuncture has long been employed as an important therapy in Chinese Medicine [4, 9]. It is believed to be efficient in curing a variety of disorders if it is appropriately used. A problem for acupuncture researchers is how they can standardize the 'appropriate use of acupuncture' scientifically and internationally.

The mechanism of acupuncture has been most studied neurologically, e.g. acupuncture's effects on the condition of pain, mainly based on pain physiology [10, 11]. With national support, a proportion of Chinese acupuncture researchers focuses its studies on the meridians, which join acupuncture points and are believed to give origin to the acupuncture effects according to the Meridian Theory of Chinese Medicine [8]. In the study presented here, we found that the stimulus in one acupuncture

point (the Jiexi) may result in the accumulation of liquid crystals in another acupuncture point (the Zusanli), which is located on the same meridian (the Stomach Meridian of Foot-Yangming) with the former.

4. Conclusion

Liquid crystals were found to appear in an acupuncture point after acupuncture stimulus in another acupuncture point located on the same meridian, and it has been demonstrated that liquid crystal detection may serve as a useful precursor method in studies of physiological phenomena. Subsequent studies of the liquid crystal materials detected suggested that they contain proteins and perhaps phospholipids, and further studies on them are anticipated employing more analytical methods. The above results shed new light on the mechanism of acupuncture.

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