Phys. Med. Biol. **54** (2009) N143–N150 doi:10.1088/0031-9155/54/9/N01

NOTE

Do acupuncture points exist?

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Received 15 November 2008, in final form 9 February 2009
Published 8 April 2009
Online at stacks.iop.org/PMB/54/N143

Abstract
We used synchrotron x-ray fluorescence analysis to probe the distribution of four chemical elements in and around acupuncture points, two located in the forearm and two in the lower leg. Three of the four acupuncture points showed significantly elevated concentrations of elements Ca, Fe, Cu and Zn in relation to levels in the surrounding tissue, with similar elevation ratios for Cu and Fe. The mapped distribution of these elements implies that each acupuncture point seems to be elliptical with the long axis along the meridian.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

There are specific acupuncture points on the surface of the human body which can be used to monitor the health of an individual and restore the health through stimulation of these points (O'Connor and Bensky 1981, Cheng 1987). The patient experiences aching or tingling sensations when these points are stimulated. These points are linked by a network of meridians which are distributed longitudinally through the body, in both human and other mammals. In traditional Chinese medicine, a life force, 'Qi', is believed to flow through these meridians. If the meridian network is disturbed such that the flow of 'Qi' is blocked, it results in illness. Each meridian relates specifically to one particular organ.

However, the existence and function of acupuncture points are a matter of controversy. Many methods have been employed to conduct research on acupuncture and its relationship

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with connective tissue structures, lymph vessels, capillaries and nerves. This structure is not only a carrier of ions and other materials but also transfers energies and messages between the cells (Langevin and Yandow 2002, Fei *et al* 1998). It has been found that there is a high level of Ca in acupuncture points by proton-induced x-ray emission (PIXE) (Dang *et al* 1997, Chen *et al* 1998). Although Ca is predominantly a skeletal element, a small proportion is found inside the cells and in extracellular fluid, acting as a chemical messenger between the cells, controlling motion and metabolism (Greenberg 1997, Theobald 2005). These researches inferred that acupuncture points were special areas where metastable Ca was conserved for use in emergencies. In this note, we investigated the chemical elements Ca, Fe, Cu and Zn in acupuncture points and in their vicinity by synchrotron x-ray fluorescence (SXRF) analysis.

2. Methods and materials

We measured the characteristic x-ray emissions of Ca, Fe, Cu and Zn at four different acupuncture points and in the surrounding tissues. The x-ray fluorescence analysis method (XRF) has been widely used for the study of human tissue samples (Theodorakou and Farquharson 2008, Geraki *et al* 2004, Börjesson *et al* 1998). PIXE and SXRF are two important methods of analysis of tissue composition (Eugene 1975, Ma and Yang 2000, Zhao *et al* 1989). PIXE uses high energy protons as a radiation source. It has been used to measure the Ca content of acupuncture points and it was found that it was much higher than in adjacent tissues. In this note, SXRF was employed for non-destructive microanalysis of several trace metal elements. SXRF is the emission of a characteristic secondary (or fluorescent) x-ray from a material that has been excited by synchrotron radiation (Valkovic and Moschini 1993, Lobinski *et al* 2006). When a material is exposed to radiation with the energy greater than its ionization energy of component atoms, the radiation can expel tightly-held electrons from the inner orbitals (lower energies) of the atom. The electrons in higher energy levels 'fall' into the lower level. At the same time, energy releases in the form of a photon, whose energy is equal to the energy difference of the two levels involved. Thus, the radiation has energy which is characteristic of the atoms present. That is to say, the synchrotron x-ray beam excites the secondary x-ray from the elements in the sample at characteristic wavelengths. It is well known that the synchrotron radiation has many advantages, such as high intensity and broad energy range. Therefore, SXRF is particularly advantageous for determining very low concentrations of various trace elements with the characteristic fluorescence x-ray being in a wide spectrum range.

We analyzed the SXRF spectrum of the characteristic fluorescence of several elements within the area of tissue exposed to the synchrotron beam using software AXIL (Van Espen *et al* 1989), in which the integrated intensities from each element were calculated and should be proportional to their concentrations:

$$N_i = k_i Q_i C_i,$$

where N_i is the integrated intensity of the ith element, Q_i is the fluorescent yield, C_i is the concentration of the ith element and k_i is a constant depending on the experimental conditions. Since several elements were measured simultaneously, the parameter k_i can be considered constant. Hence,

$$C_i : C_j : C_k = N_i/Q_i : N_j/Q_j : N_k/Q_k,$$

where i, j and k denote different elements. Consequently, the ratios of different integrated intensities from the ith, jth and kth elements corrected for their fluorescent yields can be used to derive the relative ratios of the content of the corresponding element.

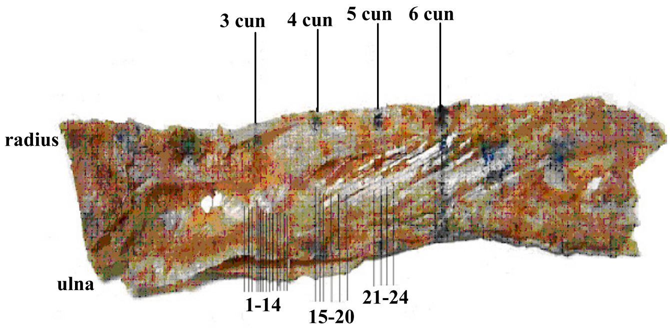

Figure 1. Picture of sample 1, the ulnar and radius periosteum and interosseous membranes of the right forearm. The black lines are the marks of the distribution of 24 points that scanned along the meridian on sample 1.

The samples investigated were provided by the Anatomy Teaching and Research Section of Second Military Medical University according to a protocol reviewed and approved by the ethics committee of the Second Military Medical University for appropriate use of human tissues. Our samples were all dissected from a cadaver by an anatomist. These samples were periosteum or interosseous membranes consisting mostly of fibrous connective tissues; hence they were very thin, less than 1 mm. The samples were always immersed in formalin. However, these samples lost their biochemical activity when they were dissected from the human body and the concentrations of trace elements in them were then kept unchanged approximately. It was examined that the formalin fixation long-term storage had little effect on most element concentrations in tissue (Bush *et al* 1995). During the sample collection, storage or treatment processes, we still try to avoid any effects which will probably make a change in the element concentrations. The same treatment processes were used for all samples in order to compare the element concentrations of different samples under similar conditions. A sample (see figure 1) was cut from the ulnar and radius periosteum and interosseous membranes of the right forearm. The whole size of the sample was approximately 10 cm (length) $\times$ 3 cm (width) (the lengthwise direction was along the meridian). It was named sample 1. The acupuncture points, Jianshi and Ximen, were located at 3/12 and 5/12 of the distance from the wrist to the elbow respectively (see figure 2). The other two acupuncture points investigated, Xiajuxu (sample 2) and Tiaokou (sample 3), were located 7/16 and 8/16 of the distance from the ankle to the knee respectively (see figure 3). A sample without an acupuncture point (see figure 3) was taken at 6/16 of this distance for reference (sample 4). The sizes of these three samples were approximately 3 cm (length) $\times$ 1.5 cm (width). All samples were taken from one individual and only one sample was collected from each site. In traditional Chinese medicine, in order to describe the position of acupuncture points a proportional unit ‘cun’ is usually used, which is a traditional Chinese unit of length and whose traditional measure is the width of a person’s thumb at the knuckle[8]. The lengths of different bodies or limbs are different. The length of 1/12 of the distance from the wrist to the elbow is one cun for sample 1 and the length of 1/16 of the distance from the ankle to the knee is one cun for samples 2, 3 and 4.

The experiments were carried out at the x-ray fluorescence station in the Beijing Synchrotron Radiation Facility (BSRF) at the endstation of a 3W1 A beam line, which had an energy range between 3.5 keV and 35 keV. A lithium-drifted silicon (Si(Li)) detector and

[8] http://en.wikipedia.org/wiki/Cun_(length).

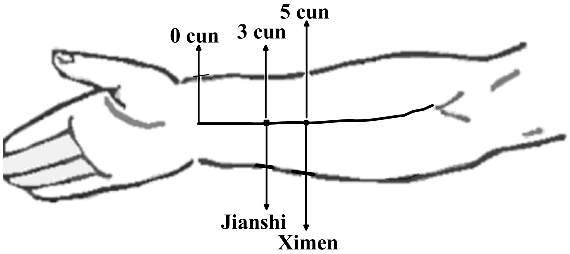

Figure 2. Location of acupoints Jianshi and Ximen. Jianshi and Ximen are located at 3/12 (3 cun in Chinese) and 5/12 (5 cun) of the distance from the wrist to the elbow, respectively, in the forearm. The word acupoint(s) is an abbreviated form of acupuncture point(s).

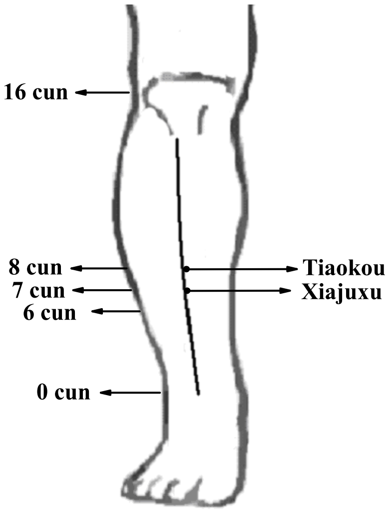

Figure 3. Sketch map of the crus with two acupoints Tiaokou and Xiajuxu. Samples 2, 3 and 4 are tissues, involving Tiaokou, Xiajuxu and no acupoint located at 8/16 (8 cun), 7/16 (7 cun) and 6/16 (6 cun) of the distance from the ankle to the knee, respectively.

spectrometer system were used to record the fluorescence spectra with an energy resolution of approximately 134 eV at 5.9 keV. The maximum counting rate was 30 000 per second. An ion chamber was used for monitoring the beam intensity. The oval beam spot size at the sample was 0.7 mm vertically and 1 mm across. 24 points were scanned along one meridian of the arm, sample 1, as shown in figure 1. We scanned the area around the acupuncture points to determine the change in the trace metal content in the vicinity of each acupuncture point. This comprised seven points along the meridian and four points perpendicular to it for sample 2, five points along the meridian and three points perpendicular to it for sample 3, and seven points along the meridian and two points perpendicular to it for sample 4. The separation of points parallel to the meridian was 1 mm and 1.4 mm perpendicular to the meridian.

3. Results and discussions

The fluorescent intensity and content of Ca were almost 20 times higher in the Jianshi acupuncture point and nearly 10 times higher in the Tiaokou and Xiajuxu acupuncture points.

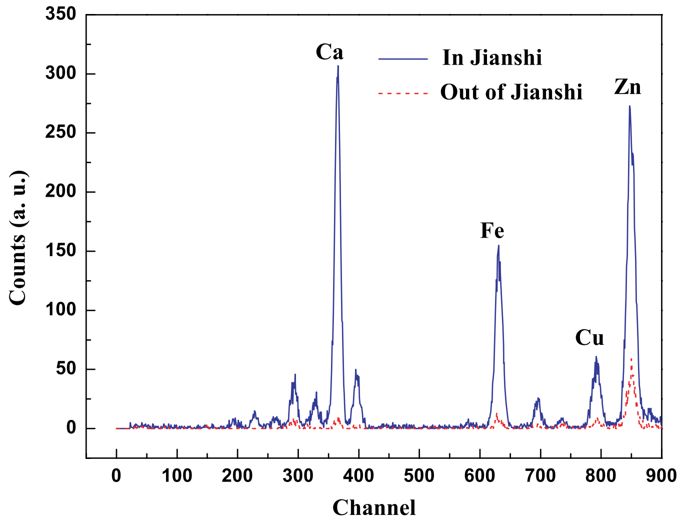

Figure 4. SXRF spectra from the Jianshi acupoint (solid line) and the surrounding tissues out of Jianshi (dotted line).

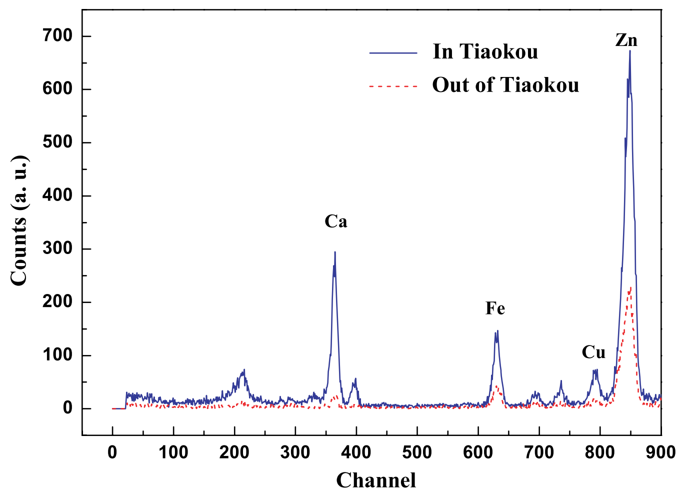

Figure 5. SXRF spectra from the Tiaokou acupoint (solid line) and the surrounding tissues out of Tiaokou (dotted line).

Of the other three trace metals, Fe, Cu and Zn, the integrated intensity from Zn was the highest, followed by Fe and Cu. Figures 4–6 show spectra collected from and in the vicinity of Jianshi, Tiaokou and Xiajuxu acupuncture points.

The parameter Q_i was taken to be 0.45, 0.33 and 0.48 for Cu, Fe and Zn respectively (Vaughan 1986, Krause 1979, Şimşek *et al* 2000). It was calculated that the ratios of concentrations of Cu, Fe and Zn (C_{Cu}:C_{Fe}:C_{Zn}) in the area of the acupuncture points are approximately 1:3.7:4.3 with a relative uncertainty of 0.17% at Jianshi, 1:3.3:17.5 with a relative uncertainty of 0.37% at Tiaokou and 1:3.5:17.9 with a relative uncertainty of 0.14%

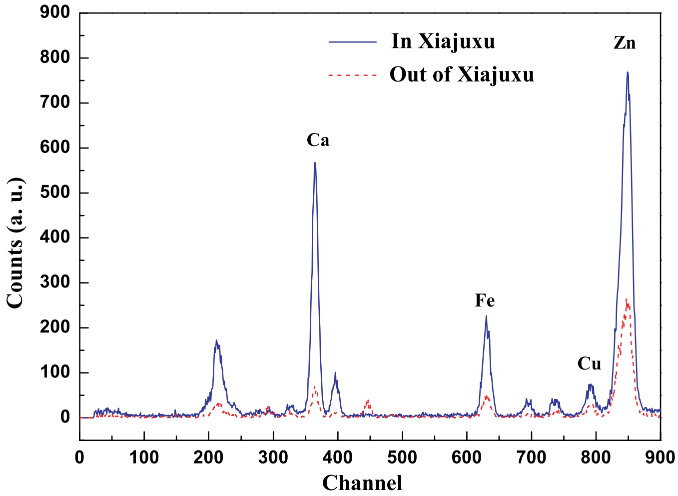

Figure 6. SXRF spectra from the Xiajuxu acupoint (solid line) and the surrounding tissues out of Xiajuxu (dotted line).

at Xiajuxu, respectively. The concentrations of Zn were different at Jianshi, Tiaokou and Xiajuxu acupuncture points but the ratios of the concentrations of Cu and Fe at all three points were approximately the same. The ratio between the concentrations of Cu and Fe may be an important characteristic of acupuncture points, since no fixed ratio for these elements was found in the surrounding tissues. The fluorescent emission depended on the position along the meridian, as shown in figure 7. The emission from the Ximen acupuncture point was very weak. It may be due to the lower element concentrations in Ximen points, but the reason for the low concentration is still not known. Except for Ximen, their contents are obviously higher than in the surrounding tissues and there are closely similar ratios of Cu to Fe at Jianshi, Tiaokou and Xiajuxu.

From the mapping spectra of samples 2 and 3 along the lines parallel to the meridian, it was found that the meridian line itself had significantly higher contents of the four metals, which gradually increased to a maximum coinciding with the acupuncture point. The full width half-maximum (FWHM) was found to be approximately 8 mm for sample 2 and 6 mm for sample 3, which coincides with the effective size of the acupuncture points. Taking the maximum as the center of the acupuncture point, the fluorescent intensities in directions parallel with and perpendicular to the meridian were mapped. It was found that away from the acupuncture point there was no fixed ratio among the contents of the four metals, in contrast to the fixed ratios found at the points themselves. Also, there were different trends in the elemental content reduction ratio $((N_{\text{center}} - N)/N_{\text{center}}) \times 100\%$ in the meridian and perpendicular to it (where N_{center} is the integrated intensity in the center of the acupuncture point and N is the intensity from any neighboring point). The ratios are shown as percentages in table 1. The uncertainties of integrated fluorescent intensities calculated using AXIL are also given. The uncertainty for Cu is large due to its very low content. The points shown are displaced from the acupuncture point by 2 mm along the meridian and 1.4 mm in the perpendicular direction. In the meridian direction, the ratio decreased less than in the perpendicular direction. These data suggest that acupuncture points are elliptical with the major axis being parallel to the meridian.

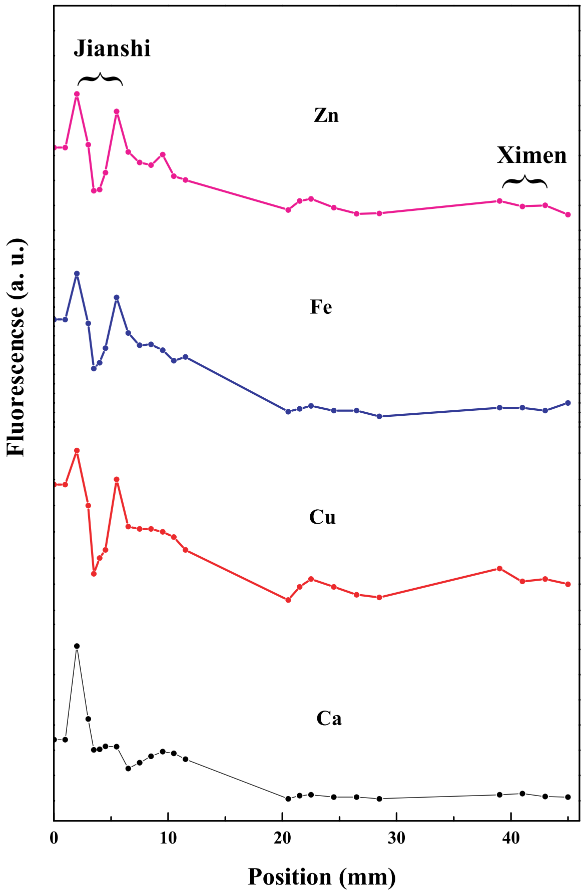

Figure 7. Contents of Ca, Cu, Fe, Zn versus the position along the meridian, where the two acupoints Jianshi and Ximen are located on sample 1.

Table 1. The reduction ratio $((N_{\text{center}} - N)/N_{\text{center}}) \times 100\%$ of Ca, Fe, Cu and Zn at a point of 2 mm away from the center of Xiajuxu and Tiaokou along the meridian and 1.4 mm away from the center of the two acupuncture points in the direction perpendicular to the meridian.

Acupuncture points	Xiajuxu		Tiaokou	
Positions of measurement	In the meridian (%)	Perpendicular to the meridian (%)	In the meridian (%)	Perpendicular to the meridian (%)
Ca	41.7 ± 2.6	79.3 ± 2.1	47.7 ± 1.8	56.6 ± 1.7
Fe	40.8 ± 4.8	71.3 ± 3.7	37.4 ± 3.7	39.2 ± 3.4
Cu	20.8 ± 11.2	86.7 ± 8.2	34.9 ± 9.5	35.9 ± 9.5
Zn	35.5 ± 1.4	40.9 ± 1.4	38.0 ± 1.5	43.0 ± 1.4

4. Conclusions

Using synchrotron x-ray fluorescence, we determined that the contents of Ca, Fe, Cu and Zn were significantly higher at three out of four acupuncture points examined than in the surrounding tissue, with closely similar ratios of Cu to Fe at points Jianshi, Tiaokou and

Xiajuxu, but not Ximen. These elements may be important in the function of acupuncture. Each acupuncture point seems to be elliptical with the long axis along the meridian.

Acknowledgment

This work is supported by the National Basic Research Program of China (no. 2006CB504509) and the Project of the State Key Program of the National Natural Science Foundation of China (Grant no. 10635060). The international ethical guidelines for biomedical research involving human subjects are abided in our study.

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