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A Preliminary X-Ray Study on Human-Hair Microstructures for a Health-State Indicator

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***Abstract*—**We present a preliminary x-ray study on human-hair microstructures for a health-state indicator, in particular a cancer case. As an uncomplicated and low-cost method of x-ray technique, the human-hair microstructure was analyzed by wide-angle x-ray diffractions (XRD) and small-angle x-ray scattering (SAXS). The XRD measurements exhibited the simply reflections at the d-spacing of 28 Å, 9.4 Å and 4.4 Å representing to the periodic distance of the protein matrix of the human-hair macrofibrous and the diameter and the repeated spacing of the polypeptide alpha helixes of the photofibrils of the human-hair microfibrous, respectively. When compared to the normal cases, the unhealthy cases including to the breast- and ovarian-cancer cases obtained higher normalized ratios of the x-ray diffracting peaks of 9.4 Å and 4.4 Å. This likely resulted from the varied distributions of microstructures by a molecular alteration. As an elemental analysis by x-ray fluorescence (XRF), the normalized quantitative ratios of zinc(Zn)/calcium(Ca) and iron(Fe)/calcium(Ca) were determined. Analogously, both Zn/Ca and Fe/Ca ratios of the unhealthy cases were obtained higher than both of the normal cases were. Combining the structural analysis by XRD measurements and the elemental analysis by XRF measurements exhibited that the modified fibrous microstructures of hair samples were in relation to their altered elemental compositions. Therefore, these microstructural and elemental analyses of hair samples will be benefit to associate with a diagnosis of cancer and genetic diseases. This functional method would lower a risk of such diseases by the early diagnosis. However, the high-intensity x-ray source, the high-resolution x-ray detector, and more hair samples are necessarily desired to develop this x-ray technique and the efficiency would be enhanced by including the skin and fingernail samples with the human-hair analysis.

***Keywords*—**Human-hair analysis, XRD, SAXS, breast cancer, health-state indicator

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I. Introduction

IN 1950, Pauling et al. first proposed the x-ray diffraction of human hairs by the structural arrangement of keratin filaments in the hair fibre [1-3]. Later Baltenneck et al. have studied on the keratinization process and the progressive organization of keratin along the follicle and the hair fibre by the synchrotron x-ray micro-diffraction [4]. They have found no significant structural difference between in vitro and in vivo grown hairs and the same structure of components in the bulb and in the fibre. For more than 10 years, there have been many groups developing methods to detect the early-stage breast cancer by using the x-ray diffraction of hair and nail samples [5-7]. In 1999, James and colleagues represented the differences in the synchrotron small angle x-ray scattering (SAXS) patterns of hair samples from breast-cancer patients compared to the healthy women [9]. With low-intensity extra rings, the SAXS alpha-keratin patterns of patients' hairs have altered from those of healthy control cases [6,7,10]. Investigated by the synchrotron-derived x-ray diffraction (XRD) of hair fibers from women with breast cancer, the x-ray diffraction patterns containing a new feature as a ring with a molecular spacing of 4.76±0.07 nm have exhibited and superimposed on the normal pattern of α-keratin [8,11,12]. Besides the standard breast imaging, these hair tests in clinical trials showed a useful assistance for the diagnosis by overlapping populations of breast cancers with the mammography detect. As further studies to understanding, the molecular mechanisms of the extra ring pattern in the breast cancer case should be illustrated [8,11,12].

In general, the significant changes in nutrient supply, inflammation, toxins, heavy metals and physical damage can result in the abnormalities in human hairs and nails [13]. Therefore, the structural quality of α-keratin filaments in the hair fibre can be applied to determine the current state of the individual's health. Recently the influence of smoking habits on the breast-cancer incidence has been preliminarily studied by using the synchrotron x ray diffraction of hair samples [14]. As synchrotron radiation for energy dispersive x-ray fluorescence (XRF) applications, the elemental distributions in human hair and bones and their quantitative analysis for Mn, Fe, Co, Ni, Cu, Zn, Br, Rb, Sr and Pb have been studied [15]. The results showed the elemental content in the samples with respect to environmental contamination, dietary habits and health status. Moreover, the concentrations of iron (Fe),

copper (Cu) and zinc (Zn) in breast tissue were investigated by a synchrotron XRF study to understand the mechanisms of breast cancer [16]. As comparison to the healthy cases, the mean concentrations of Fe, Cu and Zn for the tumour cases were higher in both types of the paired samples and non-paired samples of all patients. The tumour/healthy ratios of Fe concentrations were 1.6 and 2.7, 3.1 and 3.6 of Cu and 2.4 and 4.4 of Zn for the paired samples and for the non-paired samples, respectively. As a compromise between the complicated method and the high detection sensibility of the synchrotron x-ray radiation, the simplifier methods as x-ray diffraction and x-ray fluorescence (XRF) techniques by x-ray radiation of the available and general tools to determine the structural and elemental analyses of hair samples are extremely useful for the heath state diagnosis, particularly cancer cases.

II. Theory

A. Hair structure [4]

A single hair fiber consists of three morphological regions; a medulla, cortex, and cuticle from inside to outside, respectively. Its diameter usually varies from 50 to 90 μm. The hair is primarily composed of a fibrous structural protein as called keratin. This keratin is a same type of a protein that makes up nails and an outer layer of skin. Like other proteins in a body, the keratin protein is a large molecule of amino acids or an amino-acid residue. The amino-acid residues are periodically held together with a molecular spacing of 1.5 Å by the chemical bonds such as hydrogen bonds, cystine or sulfur bonds, salt bonds, and sugar bonds to form a polypeptide chain. This chain of human hair is known as an alpha helix or alpha keratin.

Although the hair structure is believed as a non-crystalline material, the structure of polypeptide alpha helixes is proposed as a periodical production of 3.6 amino-acid residues with a molecular spacing of 5.1-Å as a widely accepted spacing for a hair structure [6] and a repeated diameter of a alpha helix of 9.8-Å spacing. Next, about 3-4 alpha helixes are twisted together to form a protofibril as the first fibril structure of the human hair. These protofibrils are bundled together to form a 75-Å-diameter microfibril. Subsequently, hundreds of such microfibrils are grouped within a protein matrix of a repeated 28-Å spacing to produce a macrofibril. These macrofibrils by a diameter of 0.1-0.4 μm are main materials to make up the cortex layers of the hair fiber. Dead cells packed surround the cortex layers are the cuticular layers of the hair fiber (See schematics of hair fiber structure in [4]). In the center of these structures is a medullar canal for any trace metallic elements, medications and foreign leavings released from the body and some absorbed from an external environment during the hair's growth [14, 17].

B. Breast cancer [19]

In principle, breast cancer is an uncontrolled growth of breast cells. This occurs because of mutations or abnormal changes in the genes regulating the growth of cells in the breast and keeping them healthy. It is found recently that most inherited cases of breast cancer concerned with two genes: BRCA1 and BRCA2, which stands for BReast CAncer gene one and BReast CAncer gene two, respectively. Women diagnosed with breast cancer who have an abnormal BRCA1 or BRCA2 gene, often have a family history of breast cancer, ovarian cancer, or both. Identifying BRCA1 and BRCA2 is one of new techniques to detect and lower a risk for an early diagnosis. Not only these genes but also other genes, which probably playing an important role in the development of breast cancer, have been still studied in both cases of the women with and without the family history of this disease [6,19-20].

C. Changes in molecular structures of hair associated with breast cancer

A genetic abnormality or a mistake in a genetic material primarily causes a disordered growth of malignant cells or tissues. To examine changes from a normal pathological tissue could specific to a disease of that tissue [21-23]. However, some researches represent that the changes in molecular structures of tissues remote from the affected area that could be associated with the malignancy. In the case of the breast-cancer patients, the changes in the molecular structures of hair [9] and the dermal layer of skin [24] could be associated with the malignancy. For instance, specific changes in the structure of hair associated with colon cancer and Alzheimer's disease have also been reported [25,26].Recently the fiber diffraction techniques have been used to study on the muscle, collagen and keratin samples [6,9]. What appears in diffraction analysis of the different cancers is a distinctive ring, different malignancies having specific ring patterning superimposed on the normal hard alpha keratin pattern [6]. Although, the keratin pattern itself remains unaltered for hair samples from patients with cancer, the additional scattering rings by the radii lead to specify the cancer type [6,9]. In the cases with diseases as insulin dependent diabetes, the typical diffraction pattern of hair would be changed by the intensity-distribution of the meridianal arcs and the increasing radius of the intermediate filaments with respect to the helical section of the alpha keratin [18].

III. Experimental Methods

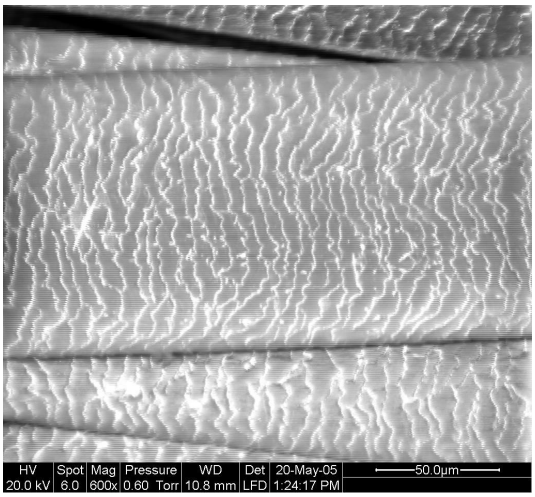
Hair samples donated from six healthy women, four women with unhealthy background and two women suffered with breast cancer for one and another with ovarian cancer, have been investigated their microstructures and element contents. So, the hair samples were categorized into three groups as P-group of patient's samples, U-group of unhealthy women's samples and N-group of healthy women's samples. To avoid a dependence of external environment, the hair samples were commonly collected from the inside site about 10-15 cm above the nape of the neck [6,8,27-29]. After washing with acetone for two times and ethyl alcohol for three times to clean and remove chemical dusts [27-29], the hair samples were cut into

small pieces of about 0.5 mm. Then the hair samples, controlled the weight of about 0.5 g, were stored in 2-cm-diameter polyethylene containers with a measuring surface covered by a thin plastic of the standard materials. In the case of small-angle x-ray scattering (SAXS) measurements, 2-cm-length hair samples were arranged one by one and slightly fixed within the sample frames.FEI Quanta 200 ESEM (Field-Emission scanning electron microscope) was used to exhibit the morphology of hair samples without a coating. Bruker Nanostar SAXS (small-angle x-ray scattering) and Bruker D8-Discover XRD (X-ray diffractometer) were employed by θ/2θ-scan measurements to determine the diffracting distance referring to hair samples' fibrous microstructures. The elemental analyses of hair samples were performed by PW2400 Philips XRF measurements.

IV. Results And Discussion

In Fig. 1(above), FE-SEM image exhibits a diameter of hair samples about 70-μm and their surface morphology as continuously overlap layers or likely a fish-scale pattern. Moreover, the structure of cuticular layers as an outside ring and the main part of cortex layers is shown in Fig. 1(below). We observed no significant difference between patient hairs and normal cases.

To analyze the fibrous structure of human-hair samples, the WAXRD measurements were employed. The results show the distinguished reflections at the d-spacing of 28 Å, 9.4 Å and 4.4 Å. When compared to the model of hair structure by Pauling et al. [4], the reflection at the 28-Å spacing most likely represents to the repeatedly protein matrix structure of macrofibrils. The reflection at the 9.4-Å spacing likely refers to a structure pattern of the alpha-helix diameter of 9.8 Å, and the reflection at the 4.4-Å distance referring to the periodic-distance of the 3.6-alpha-helix structure of 5.1 Å as mentioned above. However, this molecular spacing of 5.1 Å of the alpha helixes could be 4.5 Å when certain bonds are broken by stretching the hair samples [4].



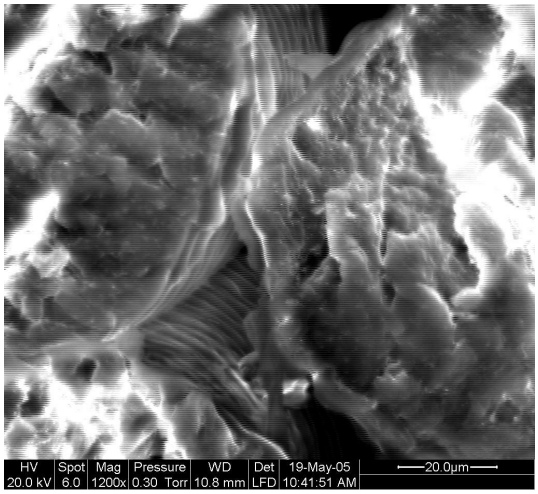


Fig. 1 FE-SEM images showing surface morphology of hair samples (above) and cross-sectional hairs (below)

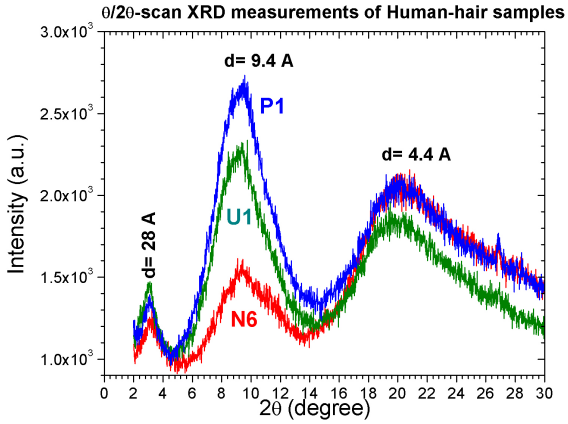
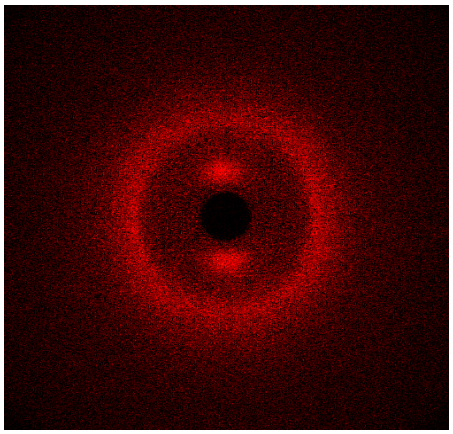


Fig. 2 θ/2θ scans of WAXRD measurements showing typical 3 distinguished reflections of hair samples

By this technique, there is no observation of extra peaks and a significant difference in 2θ-positions. This mainly results from the detection limitation of this method. However, the obvious difference in the relative intensity ratios of the reflections at the 9.4-Å spacing and the 4.4-Å spacing has revealed with higher ratios in the patient and unhealthy cases. The results indicate the mean normalized ratio of 0.964 by the patient cases, the mean normalized ratio of 0.941 by the unhealthy case and the mean normalized ratio of 0.755 by the normal case.

In addition, SAXS measurements show the distinguished x-ray scattering ring in Fig.3 (above) and the relevant reflections at the d-spacing of 9.82, 8.98 and 4.62 Å in Fig.3 (below). If the reflections of 9.82-Å spacing and 8.98-Å spacing reflections could not be separated in the case of low-resolution measurements, it would exhibit only one reflection of 9.4-Å spacing as measured in this WAXRD technique. The SAXS results are similar to WAXRD measurements: a small difference of 4.88% of the 4.4-Å-spacing reflections. This means to XRD technique enabling to find out the fibrous structure of hair. It would be better with a high-resolution and high-sensibility measuring system.



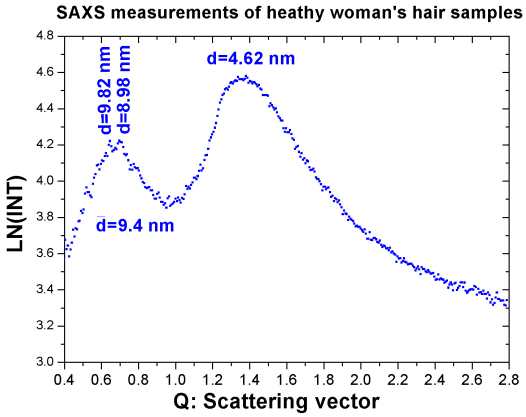


Fig. 3 SAXS images showing scattering rings of hair samples from the healthy person (above) Q-scans of the x-ray scattering (below) of the responding sample

Furthermore, XRF measurements figure out some compositional and additional elements in the hair samples such as calcium (Ca), zinc (Zn), iron (Fe), silicon(Si), and lead (Pb). The selected elements as Ca, Zn and Fe were determinded their relative amounts with a correcting a factor of each sample weight. Next, the normalized ratios of Zn/Ca and Fe/Ca were used to represent the elemental composition because of higher concentrations of Fe, Cu and Zn for the tumour tissues [16]. When compared with the normal case, higher normalized ratios of Zn/Ca and Fe/Ca were obtained in the patient and unhealthy cases as shown in Table 1.

TABLE I
NORMALIZED ZN/CA AND FE/CA RATIOS REPRESENTING TO THE ELEMENTAL STRUCTURE OF HAIR SAMPLES BY XRF

Cases	Mean norm. XRD ratio	Mean norm. Zn/Ca ratio	Mean norm. Fe/Ca ratio
Patient	0.964	0.586	0.385
Unhealthy	0.941	0.715	0.637
Healthy	0.755	0.420	0.377

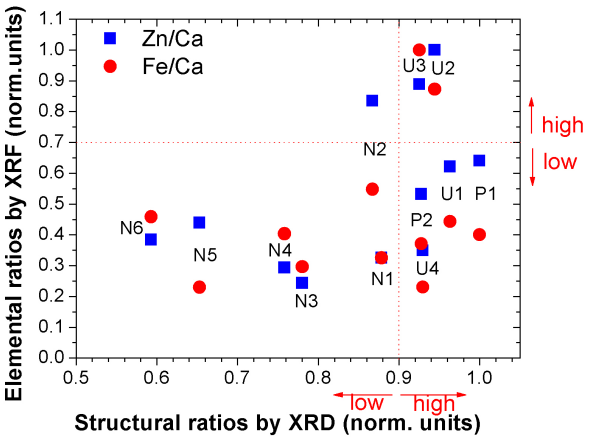


Fig. 4 The relationship between structural-analysis ratios and elemental-analysis ratios by XRD and XRF measurements, respectively

Taking account of the fibrous microstructure by XRD methods and the elemental composition by XRF measurements in Fig.4, the low normalized ratios of Zn/Ca and Fe/Ca of 0.420 and 0.377, respectively and a low normalized XRD ratio of 0.755 were obtained in the healthy cases with a few errors. In the unhealthy cases, the results were the high-normalized XRD ratio of 0.941, and the high-normalized ratios of Zn/Ca and Fe/Ca of 0.715 and 0.637, respectively with a high fluctuation. Lastly, the high-normalized XRD ratio of 0.964 and the low normalized ratios of Zn/Ca and Fe/Ca of 0.586 and 0.385 respectively were shown in the patient cases. This showed the difference in elemental composition leading to the difference in microstructures such as types, length and amount of each type. For this work, the XRD ratio could probably refer to a relation between the microstructure perfection and the Zn, Fe and Ca compositions. Although a larger hair sampling from the patients are essentially required in order to solidify this relationship between the breast-cancer diagnosis and the element and structure ratios, all measurements by x-ray techniques exhibit an uncomplicated method to indicate a health state by a human-hair microstructure.

V. CONCLUSION

We present an analysis of human-hair microstructures by an uncomplicated and low-cost method of x-ray techniques in order to indicate the health-status. This enables to be a high-promising method to diagnose the breast cancer, other cancer and genetic diseases from hair, fingernail and skin-cell samples. The early diagnosis of such diseases by this uncomplicated method would be benefit to lower a risk. The x-ray methods would be improved by using a high-intensity x-ray source and a high-resolution x-ray detector, and collecting more samples of both healthy and unhealthy cases. Analyzing skin-cell and fingernail specimens would find out the detailed correlations in the diagnosis cancer and genetic diseases.

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