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# ZINC MAPPING IN PROSTATE TISSUE USING SYNCHROTRON X-RAY MICROFLUORESCENCE

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***Resumo:*** *Many elements play an essential role in a number of biological processes as activators or inhibitors of cellular and enzymatic activity. The topographic and quantitative elemental analysis of pathologically changed tissues may shed some new light on processes leading to the degeneration of cells in the case of selected diseases. Zinc concentration in a prostate gland is much higher than that in other human tissues. The high concentration of zinc in the prostate suggests that zinc may play a role in prostate health. The aim of this work was to study the elemental distribution for Zinc in prostate tissues from patterns of relative fluorescence intensities. The measurements were performed in standard geometry of 45o incidence, exciting with a white beam and using a conventional system collimation (orthogonal slits) in the XRF beam line at the Synchrotron Light National Laboratory (Campinas, Brazil). The prostate glands were cut into pieces (slice) with thickness of 0.5 mm. The results showed the zinc distribution was not uniform for different zones of the prostate analyzed.*

***Palavras-chave:*** *X-Ray Fluorescence; Synchrotron radiation; Micro-XRF; Zinc distribution; Prostate tissue.*

## 1. INTRODUÇÃO

The knowledge of the spatial distribution of trace elements in tissues is involved in many biological functions of living organisms. These elements take part in all metabolic processes, and they are components of different enzymes, catalyzing chemical interactions in living cells. It is now recognized that there is an association between the levels of certain trace elements in human tissue and the presence of various diseases (Garg et al., 1994; Rose, 1983; Geraki and Farquharson, 2001). From these elements, zinc is a component of over 300 proteins and over 100 DNA-binding proteins with zinc fingers. Zinc is essential for proper maintenance of all cells. It is particularly important in the prostate which secretes high levels of citrate and proteins that contains zinc (Byar, 1974; Cooper and Farid, 1964).

The use of a Synchrotron Radiation X-Ray Microfluorescence (μSXRF) to determine the spatial resolution of metals and metalloids in heterogeneous biological samples has been rapidly growing (Costello et al., 2005; Koutzenogii et al., 2003; Pinheiro et al., 2003). μSXRF is an analytical technique based on the local excitation and microscopic analysis of the region of interest. This technique, in addition to displaying the concentration of the chemical elements in the material, provides, via two-dimensional images, the distribution of those elements (Lima et al., 2008). For biological

materials, the X-ray microprobe offers distinct advantages over other analytical techniques by allowing analyses to be done in situ with little or no chemical pretreatment. (Flinna et al., 2005). The intrinsic characteristics of synchrotron radiation permits to implement spectrochemical analysis with spatial resolution on the micrometer scale, high efficiency for trace elements determination and short time analysis requirement (Abraham et al., 2002). In this work, X-Ray Microfluorescence with Synchrotron Radiation was used for evaluating the elemental distribution for Zinc in prostate tissues from patterns of relative fluorescence intensities.

## 2. EXPERIMENTAL

### 2.1. Population Characteristics and Samples Preparation

This study was conducted following approval by the Internal Review Board at the Clementino Fraga Filho Teaching Hospital at the Federal University of Rio de Janeiro, Brazil. The prostate samples were collected from one sample of individual at the age of 30 years old that died from unexpected death. The samples were divided into two zones: transitional (TZ) and peripheral (PZ). Each zone was divided into two sides: left and right. After that, samples were frozen in liquid nitrogen and cut into thin slices of about 500-μm thickness. These slices were deposited on Mylar film (6 μm) and then dried in air at room temperature.

### 2.2. Experimental Organization

The measurements were carried out in the XRF beam line at the Synchrotron Light National Laboratory (Campinas, Brazil). The fluorescence spectrum was recorded with a Si(Li) detector of 165 eV FWHM at 5.9 keV in air atmosphere. The experiment was performed in standard geometry (450 x 450), exciting with a white beam and using orthogonal slits. Pixels of 300 μm x 300 μm were obtained keeping a high flux of photons on the sample. A two-dimensional scanning was performed in order to study the tendency of elemental distribution variation. Data analysis were performed by QXAS software (Bernasconi and Tajani, 1996) in order to correct the synchrotron background and fit elemental X-ray lines. The two-dimensional maps were obtained after normalization of the intensities of characteristic X-ray lines to the value of ionization chamber. The counting live time for each pixel was 6 s/step and the step size was 300 μm/step in both directions.

## 3. RESULTS AND DISCUSSION

By using the μSXRF technique it was possible to detect the following elements: P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb. Figure 1 shows a typical X Ray fluorescence spectrum of a sample of prostate tissue.

The concentration of zinc in the prostate is higher than that in any other soft tissue in the body (Bertsch and Hunter, 2001). Due to the importance of the participation of zinc in cellular processes of prostatic tissue, we focused our study in the distribution of this element in distinct zones of the prostate. The distributions of Zn in samples of prostatic tissue are shown in Figures 2-5. The relative fluorescence intensities were normalized using the maximum value found for each sample.

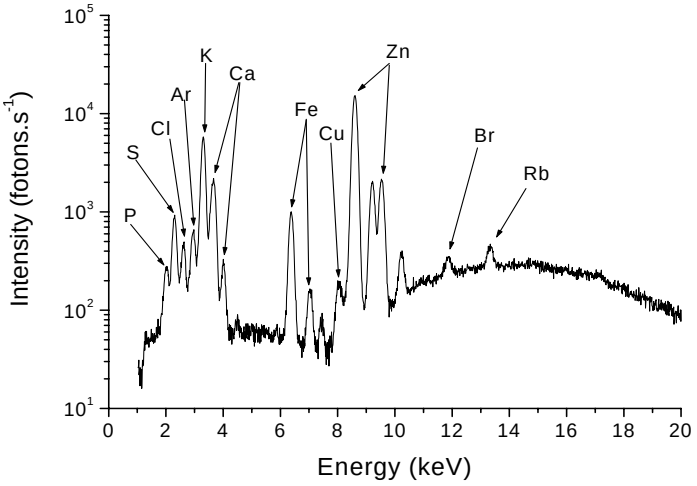


**Figura 1. The X-ray fluorescence spectra of a prostatic tissue sample.**

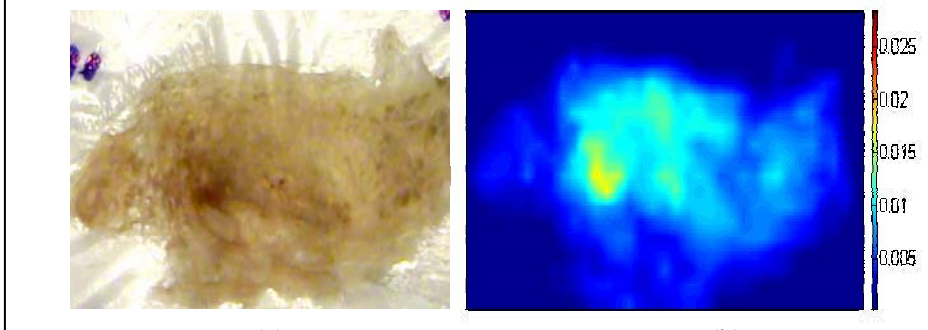


**Figure 2. Peripheral zone left.**
**(a) Photo of prostate slice, (b) image of zinc mapping.**

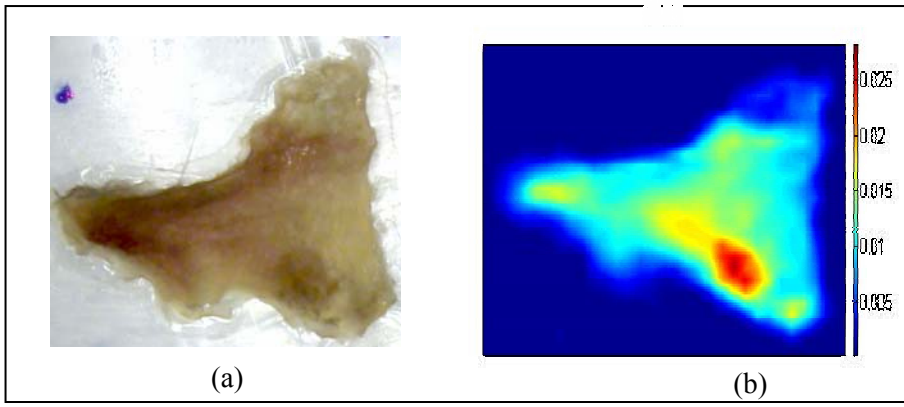


**Figure 3. Peripheral zone right.**
**(a) Photo of prostate slice, (b) image of zinc mapping.**

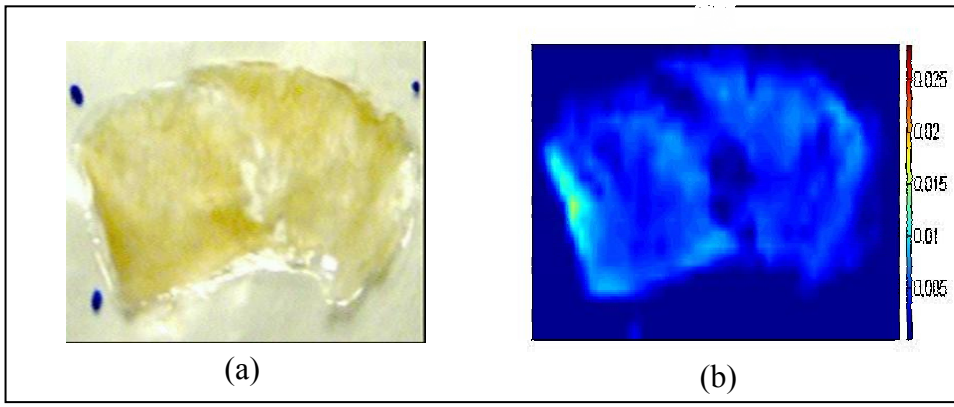


**Figure 4. Transitional zone left.**
**(a) Photo of prostate slice, (b) image of zinc mapping.**

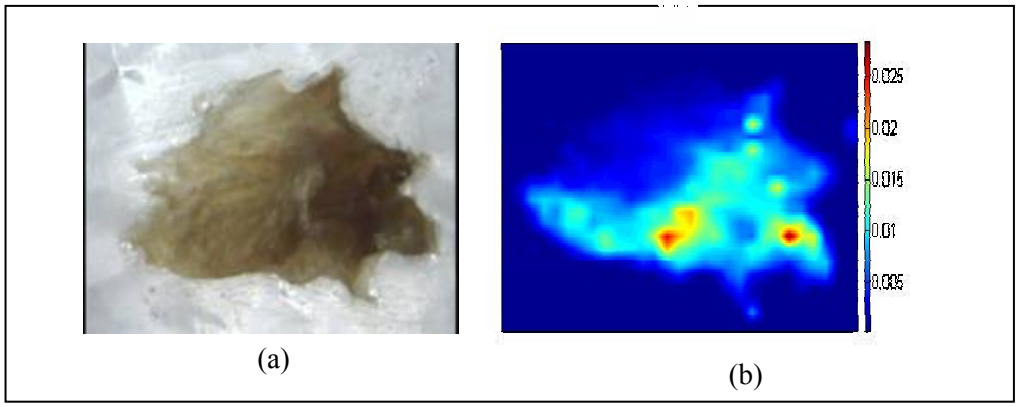


**Figure 5. Transitional zone right.**
**(a) Photo of prostate slice, (b) image of zinc mapping.**

The prostate gland in a human male is divided into three zones: the peripheral zone (PZ) covers about 70% of the gland, whereas the central zone (CZ) is comprised of 25% and the transition zone (TZ) covers the remainder of the 5%. A major function of the PZ epithelium is to secrete an extraordinary amount of citrate and the same zone accumulates about 10-fold more zinc than the rest of the gland (Byar, 1974; Cooper and Farid, 1964; Zaichick et al., 1997). The zonal anatomy is important in prostate pathology. Studies carried out by McNeal (1988) showed that most cancers develop in the peripheral zone (PZ) and benign hyperplasia mainly in the transition zone (TZ) of the prostate gland.

The higher levels of zinc are found in the mitochondria and prevent citrate oxidation by Krebs cycle. The decrease in citrate oxidation represents 65 % of the ATP efficiency (Zaichick et al., 1997; Costello and Franklin, 2006). It is well known that the concentration of zinc decreases in prostate tissue with cancer as compared to normal prostatic tissue. In prostate cancer, the malignant cells undergo a metabolic transformation from citrate-producing to citrateoxidizing cells. This occurred because of the loss of the ability of the malignant cells to accumulate zinc. The absence of high mitochondrial zinc levels removes the inhibition of m-aconitase activity. Citrate is then oxidized and the typical complete oxidation of glucose restores the efficient ATP production. For detailed descriptions of the relationships of citrate metabolism and zinc in prostate see recent reviews (Franklin et al., 2005; Costello and Franklin, 2006).

Analyzing the X-Ray fluorescence mapping in Figures 2-5, it can be observed that zinc distribution was not uniform for different zones of the prostate. The right region of the two prostate zones presented in Figures 3 and 5, showed that zinc distribution is more intense in certain areas, this is in accordance with previous studies that found that zinc distribution in the tissue prostate is not uniform (Zaichick et al., 1997; Vartsky et al., 2003). These results can be the reason for the great dispersion of zinc concentration values already described in the literature (Zaichick et al., 1997; Vartsky et al., 2003; Kwiatek et al., 2005; Yaman, 2005).

## 4. CONCLUSIONS

This study used the Synchrotron Radiation X-Ray Microfluorescence technique for mapping zinc distributions in prostate tissue. The results presented show that the technique proved to be a highly efficient to determine the elemental maps in tissue samples. The predominant element of interest for this study was zinc. It can be seen that the maps obtained for zinc was not uniform for different zones of the prostate analyzed. The results suggest that further studies on the distribution of zinc in prostate tissue must be carried out, including studies in prostate tissue with cancer and benign prostatic hyperplasia.

## 5. REFERÊNCIAS

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## 6. DIREITOS AUTORAIS