LIBPhys | Projecto de Tese de Doutoramento | Engenharia Biomédica

Sustainable X Ray Fluorescence based method for the analysis of human biopsied tissues.

Objectives:

Trace elements play an important role in biological processes and an association between the levels of trace elements and the presence of diseases such as cancer has already been established. Thus, the understanding of the mechanisms of assimilation of trace elements may be indicative of the genesis or progression of the disease. Energy Dispersive X-ray Fluorescence (EDXRF) is a spectroscopic technique that already made inroads in the analysis of human tissues.

Accurate quantitative determinations in EDXRF spectrometry, however, require the use of suitable empirical and/or theoretical methods to convert the fluorescent intensities of the spectra into the concentration of the analyte. This conversion is not straightforward as the measured intensities are strongly influenced by the surrounding elements of the sample, or matrix, resulting in absorption and enhancement effects. The existing research using this technique shows its suitability and sensitivity for these types of samples [1, 2]. However, the few published studies lack statistical representativity, so, for valid and significant conclusions to be drawn, the number of analyzed samples must be substantially higher. In this proposal, we intend to overcome this obstacle by taking advantage of the vast repository of human tissue samples, fixed in formalin and embedded in paraffin, that is stored in IPOLFG. The analysis of these tissues will allow drawing valid conclusions about the characterization and comparison of normal and tumor tissues for different organ types. However, there is a major disadvantage when using these samples, namely the type of substrate (paraffin or formalin), that increases the factor of greatest uncertainty in the quantification by EDXRF: the characterization of the dark matrix of the sample. Because paraffin and buffer formalin alter the samples' dark matrix indefinitely, it is not correct to compare

the spectra of these samples with reference spectra, as is usually done with conventional quantitative methods. So, the great advance of this proposal will be the implementation of a methodology for the accurate quantification of the elements present in paraffin-embedded human tissue samples.

This methodology will be based on the analysis and characterization of "fresh" normal tissues by the techniques already established suitable: analysis of lyophilized samples and comparison with reference materials. This way, we will obtain an accurate elemental quantification of the tissues, that we will then parameterize as we go through the paraffin inclusion steps. The final samples will be analyzed again by EDXRF, to create a matrix correction model.

This quantitative model will finally be applied to the already existing paraffin-embedded tissues and comparison of the results will be performed for tumor and normal tissues extracted from the surgical margins.

1 – Machado et al., Accuracy improvement in XRF analysis for the quantification of elements ranging from tenths to thousands μg g−1 in human tissues using different matrix reference materials, JAAS, https://doi.org/10.1039/D0JA00307G

2 – Carvalho et al., Energy dispersive X-ray fluorescence quantitative analysis of biological samples with the external standard method, https://doi.org/10.1016/j.sab.2020.105991.

Framework:

About 99% of the weight of the average human body is composed of four main elements (H, O, C and N). The remaining is made up of essential (Na, K, Mg, Ca, Cl, P, S) and trace (Mn, Fe, Cu, Zn, Se) elements. Physiochemical properties of trace metals govern their uptake, intracellular distribution, and the binding of the metal compounds in biological systems. This way, inadequate or lack of trace elements as enzyme cofactors exposes the individual to carcinogenic stress. Considering the worldwide increasing prevalence of cancer and the possible contribution of trace elements to carcinogenesis reported in some studies, it is pertinent to analyze trace element content in healthy and cancerous tissues. Thus, the quantification of these elements may constitute a pioneering alternative method of diagnosis: a sensitive and accurate method of identification that allows, for example, distinguishing, in situ, normal from tumor tissue in order to provide for a more

conservative approach to healthy tissue removal during surgery. This proposal will allow to establish possible correlations between the various elements and factors like age, sex, tissue origin and stage of disease, leading to a better understanding of carcinogenesis.

Tasks and timeline:

The proposal is divided in 4 main tasks that will occur concurrently:

Task 1 (6 months) – Collection of tissues to build a database of reference materials with a variable elemental composition - In order to accomplish this, we will make use of "fresh" tissue, from different organs to be analysed using the benchmarked methodologies – lyophilization, powdering and pelletizing of the samples and analysis using EDXRF and external standard method quantification.

Task 2 (6 months) – Evaluation of the impact of the tissues' conservation medium - With this task, we aim to understand if the preservation process affects the tissue with respect to trace element concentration. To make sure that the procedure does not affect the EDXRF spectra, comparisons between preserved tissues and fresh tissues will be performed. In the sample collection of task 1, samples of the same tissue will be collected and frozen at -80 ºC while other samples from the same piece will be placed in a preservation substance. A study to check if there is a preservation time dependence of the samples elemental concentration will also be performed.

Task 3 (18 months) – Development of a model for the parametrization of the paraffined samples - This task aims to identify a parametrisation that would allow us to extract the element concentration of trace elements from tissue samples embedded in paraffin. For this purpose, many approaches of data analysis computational methods will be considered (frequentist approach vs Bayesian statistic approach). But the applied parametrisation will strongly depend on the amount of data that we can obtain and on the actual comparison between the spectra of paraffin embedded samples and with fresh samples.

Task 4 (24 months) – Sample analysis and statistical assessment - After the development of an accurate quantification methodology, we will collect paired

samples of tumour and normal tissues from IPOLFG to obtain the quantitative results for different organs and stages of the disease.

Location:
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