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An improved neutron autoradiography set-up for ^{10}B concentration measurements in biological samples

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ABSTRACT

Aim: Boron Neutron Capture Therapy (BNCT) is a binary hadrontherapy which exploits the neutron capture reaction in boron, together with a selective uptake of boronated substances by the neoplastic tissue. There is increasing evidence that future improvements in clinical BNCT will be triggered by the discovery of new boronated compounds, with higher selectivity for the tumor with respect to clinically used sodium borocaptate (BSH) and boronophenylalanine (BPA).

Background: Therefore, a ^{10}B quantification technique for biological samples is needed in order to evaluate the performance of new boronated formulations.

Materials and methods: This article describes an improved neutron autoradiography set-up employing radiation sensitive films where the latent tracks are made visible by proper etching conditions.

Results: Calibration curves for both liquid and tissue samples were obtained.

Conclusions: The obtained calibration curves were adopted to set-up a mechanism to point out boron concentration in the whole sample.

1. Background

Boron Neutron Capture Therapy (BNCT) is a binary hadrontherapy consisting in the administration of a boronated compound like boronophenylalanine (BPA), which concentrates more ^{10}B in tumor than in healthy tissue. After boron administration the tumor site is irradiated with low energy neutrons, causing neutron capture reactions in boron. This nuclear interaction releases two high LET particles (an alpha

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particle and a lithium ion) that deposit all their energy inside the cell. BNCT can thus deliver a therapeutic dose to the tumor while sparing the healthy tissue, triggering and possibly achieving tumor control.

The development of new ^{10}B delivery systems, with higher selectivity for the tumor with respect to clinically used sodium borocaptate (BSH) and boronophenylalanine (BPA) will trigger future improvements in clinical outcomes of Boron Neutron Capture Therapy.[1] A ^{10}B concentration measuring technique for biological samples is needed in order to evaluate the performance of new boronated formulations. At the Triga Mark II nuclear reactor in Pavia, two techniques were developed: Alpha Spectrometry (AS)[2] and Quantitative Neutron Capture Radiography (QNCR).[3] The latter was recently improved to ensure higher accuracy and optimized efficiency when a high number of samples is analyzed.

In the first QNCR set-up, described by Gadan et al.,[3] a suitable calibration curve and a sufficient resolution were achieved; however, there was still potential for improvements. Firstly, it was necessary to reduce the time of the overall procedure, determined by an etching time longer than 2 h. This would increase the time efficiency of the set-up and possibly open the road to quasi-online blood borne boron concentration measurements. Secondly, in order to reduce the background signal, a better selection of the tracks left on the Solid State Nuclear Track Detector[b] (SSNTD) by ^{7}Li and α particles had to be achieved. The latter condition would simplify data acquisition, avoiding the implementation of a complex morphological track selection algorithm that was previously necessary to reject the tracks due to protons.[c] Furthermore, this would improve the resolution of the measurement. These goals were reached employing PEW40 ($KOH + C_2H_5OH + H_2O$) as a chemically etching solution at 70 °C, instead of the NaOH solution previously used. This set-up decreased the etching time from 2 h to 10 min. Moreover, only tracks from ^{7}Li and α ions are thus detected, decreasing by consequence the relative error of the calibration from 7% at 1σ of C.L to 5% at 1σ of C.L.

2. Aim

To further exploit the features of autoradiography, another QNCR technique was set up to perform a quantitative imaging of the ^{10}B distribution in a biological tissue sample.[5] These images can then be compared to histological preparation of contiguous sections in order to have a proof that ^{10}B concentrates in the tumor with respect to the normal tissue. This quantitative imaging was attained by merging adjacent pictures throughout a scan of the area where the sample was fixed on the SSNTD. The tracks of each image were then analyzed with the QNCR set-up reported in Section 2.4. Consequently it was possible to reassemble the image of the whole sample outlining the ^{10}B concentration distribution.

The QNCR set-up was then validated by comparing the outcome of boron concentration measurements of the same sample with alpha-spectrometry (AS) results.[2] Moreover, through a collaboration with a group working at the Comision Nacional de Energia Atomica (CNEA, Argentina), tissue and cell samples treated with BPA (following a routine administration protocol) were measured by QNCR and AS in Pavia and by ICP-AES, ICP-MS and QNCR with different films in Buenos Aires. Although the project is in an early stage, results show that all the cited techniques are consistent for ^{10}B concentration measurements.

This improved neutron autoradiography method was then applied to ^{10}B concentration measurements in tissues from small animals and cell cultures treated with new carriers, within the framework of the BNCT feasibility study for osteosarcoma.[6] The experiments were carried out testing three categories of carriers: gold nano-particles, liposomes, polymeric nano-particles and BPA as a reference. In particular, ^{10}B loaded liposomes and BPA were administered to Sprague-Dawley rats bearing osteosarcoma. After treatment, healthy muscle and tumor mass were explanted and prepared for QNCR and AS. The results concerning boron biodistribution obtained in these tissues are presented and discussed here. Boron biodistribution obtained in vivo will be used in the following part of the experiment, consisting in in vivo irradiation of rats with osteosarcoma to test BNCT efficacy in tumor remission and BNCT toxicity for healthy tissues.

3. Material and methods

CR-39[d] was used as SSNTD, as described in Gadan et al.,[3] thus, before proceeding with boron concentration measurements, the bulk etch rate associated with the new etching method was characterized as a comparison to the previous set-up. Subsequently the most favorable irradiation conditions were chosen. Calibration curves were then built both for liquids and tissue samples. These results were then used for boron concentration measurements of tissue samples from animals administered with different boronated substances. The same samples where then analyzed with the AS method,[2,6] thus validating this new QNCR set-up.

Subsequently, the calibration curves were employed to develop a c++ program in the ROOT[7] framework to recombine the microscopic images resulting from a scan of the whole irradiated tissue sample. The output of the software was a macroscopic boron concentration map.

3.1. *Bulk etch rate measurement*

To characterize the track formation in a SSNTD, as extensively described in 3, the first step consists in the evaluation of the bulk etch rate V_b.

V_b was measured and compared to the one obtained by Gadan et al.,[3] thus highlighting the differences of the etching conditions in the two setups. For V_b measurement, the CR-39 films were etched for the following times: 0, 2, 4, 8, 10, 20, 30 and 60 min, in a PEW40 solution at 70 °C, which was obtained

[b] These kinds of detectors were recently used to display boron at cellular level [4].

[c] In biological tissues protons with an energy of 590 keV are produced by the neutron capture reaction on nitrogen: ^{14}N (n,p)^{14}C.

[d] Rectangular polyallyldiglycol carbonate (PADC), from Intercast Europe manufacturer, with 75 mm × 25 mm area and 1 mm width.