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A N I N T E R N A T I O N A L J O U R N A L

Evaluation of Hela Cell Lineage Response to β Radiation from Holmium-166 Embedded in Ceramic Seeds

Eduardo Sarmento Valente[1*], Ethel Mizrahy Cuperschmid[2] and Tarcisio Passos Ribeiro de Campos[2]

[1]Centro de Desenvolvimento da Tecnologia Nuclear; Av. Presid. Antônio Carlos, 6627; 31270-90; Belo Horizonte - MG - Brasil. [2]Escola de Engenharia; Universidade Federal de Minas Gerais; Av. Presid. Antônio Carlos, 6627; 31270-901; Belo Horizonte - MG - Brasil

ABSTRACT

This work studied the effects of β radiation of Ho-166 embedded in ceramic seeds on HeLa cells. Methodology consisted in the production of ceramic seeds with holmium-165 by sol-gel route. Chemical and physical characterizations of the seeds were performed. Subsequently, nuclear characterization was performed by gamma spectrometry. Experimental and theoretical activities were defined and initial dose rate were evaluated by MIRD (Medical Internal Radiation Dose Committee) methodology. The seeds were placed in confluent culture flasks and remained for six radionuclide half-lives. Biological results were represented by a clean 6 mm diameter area around the seed where the tumour cells were killed. The initial dose rate was 15.5 Gy. h^{-1}. The maximum absorbed dose was 591.3 Gy. The features of the Ho-166 seeds suggested that such ceramic seeds were suitable for high dose rate brachytherapy.

Key words: Brachytherapy, ceramic seeds, holmium, cancer control, HeLa cells

INTRODUCTION

Malignant neoplasia is even today a public health problem. Despite the efforts of scientists and researchers, many neoplasic occurrences remain uncontrolled, resulting in approximately seven million deaths annually worldwide (Buono et al., 2007). Recent scientific advances in diagnosis and control have not been sufficient to provide a cure for the cancer, although remission occurs in many cases. Long term control with normal quality of life is the primary goal in cancer research (Dow et al., 1996).

Radiotherapy plays an important role in the control of neoplasia, either as a single therapy, or in addition to surgical treatment and chemotherapy. Radiation therapy destroys the cancer cells by exposing them to ionizing radiation delivered to the tumour bed. It can be used as an adjuvant therapy in postoperative treatment to eliminate the residual neoplasic infiltrations. Radiation can also be used as a primary therapy to control an unresectable tumour, when its complete removal cannot be accomplished due to the need to preserve the organic functionality of the human body. Radiation therapy has already proven effective against the tumours, and is currently the most widely used technique in treating them. Two modalities, teletherapy and brachytherapy, in addition to surgery and chemotherapy, account for

[*]Author for correspondence: valente@cdtn.br

almost all types of treatment (Hortobagyi et al., 1988).
Teletherapy applies a radiation beam from outside the patient's body toward the tumour, delivering a fractional dose of 1.8 to 2.0 Gy. The treatment usually takes place over a period of weeks, depending on the total dose, how it is fractioned, the tumour site and its response to radiation. (Livi et al., 2005).
Intracavitary brachytherapy is a technique for tumour control in which radioactive sources are placed as close as possible to the tumour, using the natural body cavities as a pathway. In interstitial brachytherapy, the radioactive source is placed in the tumour interstices. The implantation of radioactive sources can be temporary or permanent. The permanent implant mode uses sealed radioactive sources, incorporating I-125 radionuclide, for example, encapsulated in thin titanium tubes (Blank 2000).
Ceramic seeds synthesized by sol-gel route have been developed by researchers of the NRI-UFMG (Núcleo de Radiações Ionizantes – Universidade Federal de Minas Gerais) group and proven suitable for the purposes of interstitial brachytherapy (Roberto et al., 2003). They are produced in small dimensions, are chemically stable in simulated body fluid (SBF) and in *in vivo* experimental implants (Silva et al., 2005). Also, it is easily activated in low neutron fluxes (Valente and Campos, 2007).
This work evaluated the response of HeLa cells to β radiation emitted by the radioisotope Ho-166, embedded in a ceramic matrix as small seeds.

MATERIALS AND METHODS

Ceramic synthesis

A set of seeds synthesized by the sol-gel technique and incorporating Ho, composed of SiO_2, Ho_2O_3 and CaO was produced. The nuclide precursor Ho-165 was provided by $Ho(NO_3)_3$, supplied by Alfa Aesar (Johnson Mattehy Company) with 99.8% purity. These seeds were chemically characterized by gamma spectrometry after being irradiated in a TRIGA (Training, Research and Isotope – General Atomic) type research reactor IPR-R1@100 kW. Induced activity and Ho concentration were measured. In addition, the activity of all possible radionuclides on the seed was investigated.

Physical characterization

From the set of the seeds, dimensional characterization was performed and stereoscopic images were made. The weight in mg was measured on an electronic balance. Density was determined from these data.

Chemical characterization

In the preparation of ceramic seeds by the sol-gel technique (Brinquer and Scherer, 1990), natural holmium was added in order to achieve a 20% concentration in weight on the ceramic. As the sol-gel technique involves physical transformations to the material, it was important to verify the final concentration of Ho in the seeds.

Reactor irradiation and theoretical and experimental activity measurements

A set of the seeds containing Ho-165 was irradiated in a TRIGA type, 100 kW research reactor, IPR-R1 with neutron fluxes of 2.8×10^{12} n/cm².s (thermal) and 2.6×10^{11} n/cm².s (epithermal) (Zangirolami et al., 2010). It is important to consider the epithermal neutron flux in induced theoretical activity evaluation because the neutron absorption cross section for Ho-165 in this range of energy is higher than that for thermal neutrons. The induced activity was, therefore, obtained to a large extent through the epithermal neutron flux. The induced seed activity is given by the expression number 1 (Parry, 2003):

$$A_0=[(m.\Theta.N_A)/A].[(\sigma_\gamma.\Phi_{th})+(I_\gamma.\Phi_{epi})].(1-e^{(-\lambda.t)}).e^{(-\lambda.d)}\ Bq \quad (1)$$

where *m* is the mass of the element to be irradiated in grams, the factor Θ is the isotopic abundance of the target nucleus, *A* is the atomic mass of the element, N_A is Avogadro's number, Φ_{th} is the thermal neutron flux, Φ_{epi} is the epithermal neutron flux, σ_γ is the neutron capture cross section of the target nucleus in cm², I_γ is the resonance integral for the epithermal neutron flux of the target nucleus in cm², λ is the decay constant of the produced radionuclide, *t* is the time of irradiation in seconds and *d* is the time to decay after irradiation in seconds.

β particle range

The range of β particles from Ho-166 determines the volume in which all β particle energy will be released. It is possible to calculate the range through the empirical mathematic expression number 2, considering water as a medium (Evans, 1955):

$$R_{ex}\ (cm) = 0.412E^{(1.265 - 0.0954 \ln E)}/\rho \qquad (2)$$

in which R_{ex}, is the extrapolated range in cm, E is the maximum energy of β particle in MeV and ρ is the density of the medium in g/cm³.

Total dose estimation

The dose rate and total imparted dose due to the β particles within the volume of water delimited by its range were calculated according to the MIRD (Medical Internal Radiation Dose Committee of the Society of Nuclear Medicine) methodology (Cember, 1989). Dose values help to determine if the seed is capable of eliminating the cancerous cells.

Cell lines

The tumour cell lines used were HeLa from cervix carcinoma (ATCC CCL2). The cells were grown in DMEM (Dulbecco's Modified Eagle Medium). The cultures were maintained at 37ºC in the DMEM, supplemented with 10% fetal calf serum and 4% glutamine, under a 5% CO_2 atmosphere. The nutrient medium was changed every 72 h and the cells were divided into new flasks at a density of 30,000 cells/cm^2, as soon as they reached 100% confluence. The experiments were performed on a T-25 flask, completely covered by cancerous cells. Gentian violet was used to color and fix the cells and fragments on the flask (Reitzer et al., 1979).

Cell culture and irradiation

Ceramic seeds with radioactive Ho-166, with different activities, were laid over the HeLa cells culture grown in laboratory flasks to evaluate the response of these cells to β radiation. An arrangement of three and one of four seeds with different activities were placed in polymeric capillary tubes spaced by stainless steel wires in an acrylic holder as shown in Figure 1. This arrangement was necessary because of the possibility of change in the seed's position as a result of handling the flasks, or floatation in the culture medium. This tendency to float and move in liquid nutrient had been observed during previous experiments.

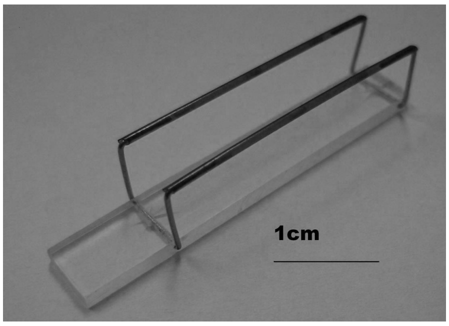

Figure 1 - Acrylic spacer used in the tumoral HeLa cells experiment.

RESULTS

Physical characterization of the seeds

The synthesis of ceramic materials by sol-gel route was basically on a trail and error basis, hence, several attempts were required until a suitable geometry was obtained. The appearance of the seeds after synthesis is shown in Figure 2.

The physical characteristics of seeds are shown in Tables 1 and 2:

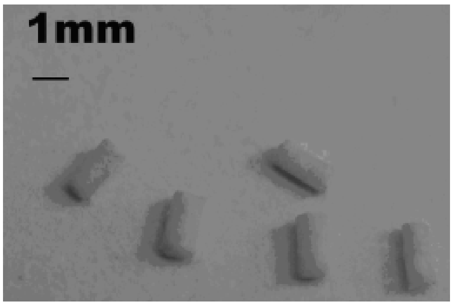

Figure 2 - Seeds with holmium after synthesis by sol-gel route.

Table 1 - Average dimensions of the seed.

Average diameter (mm)	Average length (mm)
0.75±0.06	1.62±0.08

Table 2 - Average mass and density of the seeds.

Average mass (mg)	Average density (g/cm^3)
1.81±0.09	2.52±0.12

Gamma spectrometry and holmium concentration

Instrumental Neutron Activation Analysis (INAA) and Atomic Emission Spectrometry with Coupled Induced Plasma (ICP-AES) were used to quantify the mass relation of the radioisotope Ho-166 in the seeds. Other detectable elements did not attain the minimum level of activity required for the analysis, indicating that they were only present in trace amounts. This result was very useful as the presence of other radioisotopes in the seeds was not desirable. Gamma radiation spectra, taken on a gamma spectroscopy system coupled to an HPGe detector, are shown in Figures 3 and 4.

The result of the weight concentration of Holmium in the ceramic seeds determined by neutron activation analysis, K_0 method, is shown in Table 3.

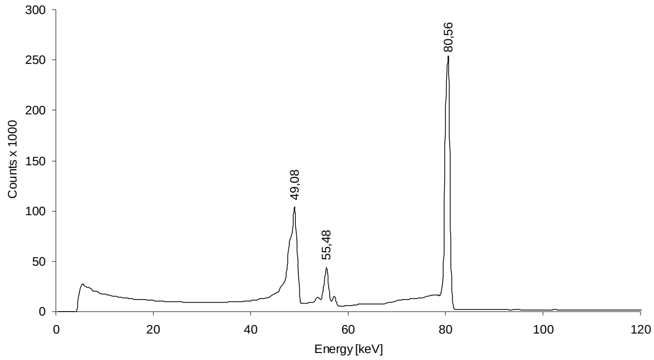

Figure 3 - Low energy gamma radiation spectrum of Holmium-166.

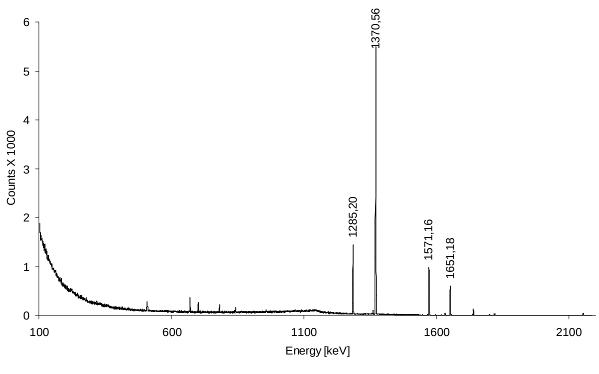

Figure 4 - High energy gamma radiation spectrum of Holmium-166.

Table 3 - Holmium concentration in weight in ceramic seeds measured by INAA, K_0 method.

Sample	Concentration (%)
Ho-1	20.9±0.7
Ho-2	20.2±0.9
Ho-3	21.5±0.5
Ho-4	20.7±0.5

Induced activity evaluation

Ceramic seeds with Ho were irradiated for eight hours in the central thimble of the TRIGA type research reactor IPR-R1. The activity was measured one hour after the irradiation, with a CAPINTEC® dose calibrator, model CRC-25, used in nuclear medicine. The results are presented in Table 4. Theoretical activity was also evaluated by expression number 1 and shown in Table 5.

Table 4 - Experimental activity of the seeds with holmium irradiated for 8 h in the central thimble of nuclear reactor IPR-R1.

Measured activity per seed in MBq (mCi)
132.1±2.6 (3.57±0.07)

Table 5 - Theoretical evaluated activity of the seeds.

Calculated activity per seed in MBq (mCi)
93.2±1.8 (2.52±0.06)

The difference observed between the calculated and measured values of the activity of seeds was possibly due to factors such as (i) uncertainty in detector-source geometry during the activity measurement, (ii) uncertainty in the seed's position in the central thimble of the reactor during irradiation, exposing them to a neutron flux different from that adopted in the calculation, and, (iii) the influence of other absorbing samples close to the central thimble of the IPR-R1 reactor.

Range of β particles emitted by Ho-166

The maximum range of β particles emitted by Ho-166 was evaluated according to expression number 2, assuming a maximum energy of 1854.7 keV. Thus, the mass extrapolated range R_{ex} in g/cm^2 was 0.867 g/cm^2 and the range in water was 8.67 mm, given a density of 1.0 g/cm^3. Thus, the volume in which the radiation dose would be delivered due to β particles corresponded, approximately, to a sphere with an 8.7 mm radius.

Evaluation of dose rate and total absorbed dose in water

The dose rate and total dose imparted were calculated according to MIRD methodology (Cember, 1989). It takes into account the sum of the product of the average energy of the beta particle multiplied by its yield divided by the mass of the volume defined by its maximum range. The following data were used in the calculation: initial activity A_0 of 37 MBq (1 mCi) and R_{Ho} as 8.7 mm, for Ho-166 seeds. The energies and yields of the major beta particle emission from Ho-166 are 1854 keV (50%) and 1773.1 keV (48.7%).

Thus, the initial average dose rate produced by those high energy β particles emitted by Ho-166 was 5.18 Gy/h. The total dose deposited in water during six half-lives by two higher energy β particles from Ho-166 was 197.2 Gy. Similarly, for an Ho-166 seed with initial activity A_0 of 111 MBq (3 mCi) and range R_{Ho} of 8.7 mm, the initial average dose rate produced by two high energy β particles from Ho-166 was 15.5 Gy/h. Thus, the total dose deposited in water during six half-lives by two higher energy β particles from Ho-166 was 591.3 Gy.

Response of tumoral HeLa cells to β radiation

The cell lines used in the present study were grown in a monolayer attached to the bottom of the flask. The irradiation of the tumour cell bed during five half-lives of Ho-166 led to a gradual decrease in the number of cells around the seed, producing a halo. These halos were clearly visible after dyeing the cells. These attached cells which received radiation doses were probably driven into apoptosis or necrosis and detached from the flask.

The result of the experiment is shown in macroscopic photographs of the culture flasks (Fig. 5 - 8). There was a disc, namely the clean zone, with a diameter of approximately 6 mm around the central location of each seed. As shown in the pictures, in the centre of this clean zone, a small blue spot appeared where the seed has been placed.

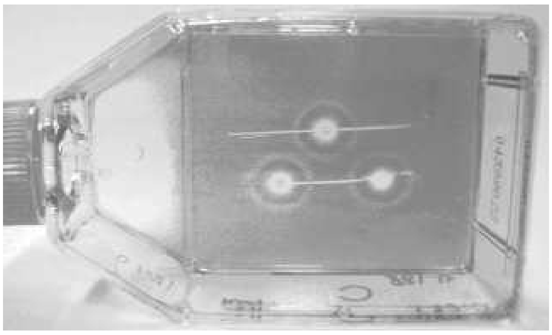

Figure 5 - The T-15 flask after three 37MBq (1mCi) Ho-166 seed irradiation, with spacer withdrawal and cell fixed and dyed.

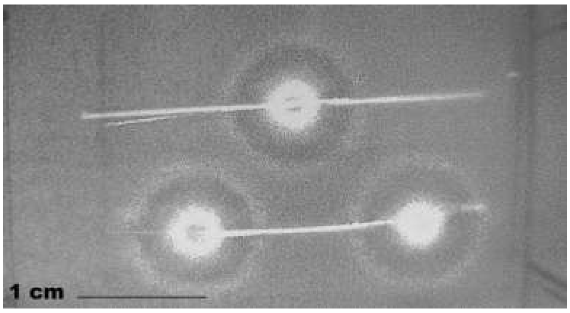

Figure 6 - Amplified details of the HeLa cell culture response after irradiation with three seeds at 37 MBq (1 mCi) activity per seed.

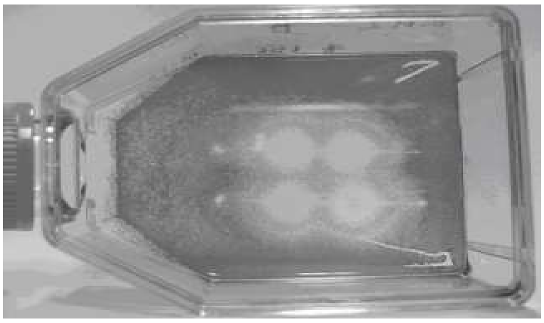

Figure 7 - The T-25 flask after Ho-166 seed irradiation, following withdrawal of spacer, in which Ho-166 seeds were held with 111 MBq (3 mCi) per seed.

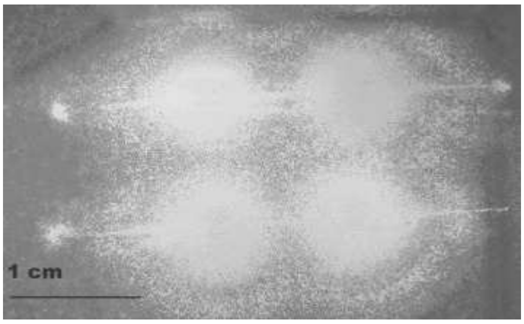

Figure 8 - Amplified details of the HeLa cell culture response after irradiation with four seeds at 111 MBq (3 mCi) activity per seed.

On all the areas of the clean zone, the cells were killed by the radiation and detached from the flask by natural shedding. The cells whose death was induced lost their ability to produce adhesion protein and thus detached off the bottom of the culture flask. Outside of the clean zone, there was a blue ring composed of cells that remained attached to the flask at that time of exposition. They seemed to be undergoing necrosis, and thus had different shape. In this state, they effectively retained the coloured dye more strongly.

The analysis of the samples showed cell debris adhered to the bottom of the flask, located in the centre of the clean zone as mentioned before. They originated from the deteriorated cells that were very close to the seeds. This region received a high dose of radiation, and the cells were probably killed by necrosis, after damage to all their cellular structures. The high level of dose in this region, theoretically evaluated, seemed to confirm this hypothesis.

The cells located at an intermediate distance, in the clear zone, were probably killed either by the apoptosis, caused by the damage to the structure of their DNA (Hall, 1993; Ballarini, 2010) or by necrosis, while other cellular structures, such as the cytoplasmatic membrane, were damaged by the radiation. The dark ring around the clean zone exhibited a large number of swollen cells among the cells of normal appearance, but in smaller amounts. These swollen cells were probably undergoing necrosis. They received a low radiation dose compared to the innermost region because this area was at a distance that few β particles reached. It was observed that these cells, even in possible process of necrosis, were still adhered to the flask bed.

DISCUSSION

The physical characteristics of the seeds were adequate both in shape and size that permit implants to be administered using hypodermic needles fitted with a long plunger. Smaller seeds have also been produced and may be used with smaller needles, reducing the effect of needling of the tumour. The ceramic seeds were conceived to be implanted using the same technique used for metallic I-125 seeds.

With regards to the radioactive characteristics of the seeds, it seemed that β emitting elements embedded in a ceramic matrix were suitable for brachytherapy as demonstrated by the experiments with HeLa cells. The maximum beta range goes up to 8-10 mm diameter and the activity of 111MBq (3 mCi) per seed is enough to induce the death on HeLa cancerous cells supposedly by necrosis and apoptosis. The theoretical β particle range from Ho-166 will allow spacing up to 14 mm in near future implants *in vivo* experimental trials. Compared with metallic I-125 seeds, this spacement will allow covering the same volume of tumoural tissue with a smaller number of the seeds.

The spherical volume restricted by a radium equivalent to the range of β particles of Ho-166 in water is high enough to provide brachytherapy at a high dose rate (HDR), differently from the low dose rate (LDR) provided by metallic I-125 seeds.

The *in vitro* response of HeLa cancerous cells was macroscopically identified by the presence of a region of total cell death around the seed, surrounded by a region of cells displaying signs of imminent death. If the same effect were to occur in real tumours, their growth would be controlled or stopped.

CONCLUSIONS

The use of Ho-166 in brachytherapy is very attractive due to its nuclear characteristics that are *β* particle emission with maximum energy of 1855 keV, and 80.5 keV gamma radiation emission and half-life of 26.8 hours. The calculated range of β particle was compatible with the size of the halos found in the experiments. It was observed that the biological effects of radiation in HeLa cells culture went beyond the range of β particles. These effects are to be investigated together with the effects of the gamma radiation that, in addition to β particles, contribute to the total dose delivered to the tumour cells. For these reasons, it is expected that ceramic seeds could provide high dose rate in the tumour and reduce the treatment time. The evidences showed in this work led to the conclusion that the research should advance although the aims of this work were totally achieved concerning to the study of the effects of β radiation over HeLa cells. The next step would be to investigate the radioactive seed's effects on other tumoral cells lineage. Further studies *in vivo* might demonstrate the efficiency of this brachytherapy as well as permit the adjustments of the activity that could guarantee the application of therapeutical radioactive dose on tumours.

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