Calibration of GafChromic EBT3 for absorbed dose measurements in 5 MeV proton beam and $^{60}\text{Co}$ $\gamma$-rays

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Purpose: To study EBT3 GafChromic film in low-energy protons, and for comparison purposes, in a reference $^{60}\text{Co}$ beam in order to use it as a calibrated dosimetry system in the proton irradiation facility under construction within the framework of the Oncological Therapy with Protons (TOP)-Intensity Modulated Proton Linear Accelerator for RadioTherapy (IMPLART) Project at ENEA-Frascati, Italy.

Methods: EBT3 film samples were irradiated at the Istituto Nazionale di Fisica Nucleare—Laboratori Nazionali di Legnaro, Italy, with a 5 MeV proton beam generated by a 7 MV Van de Graaff FN accelerator. The nominal dose rates used were 2.1 Gy/min and 40 Gy/min. The delivered dose was determined by measuring the particle fluence and the energy spectrum in air with silicon surface barrier detector monitors. A preliminary study of the EBT3 film beam quality dependence in low-energy protons was conducted by passively degrading the beam energy. EBT3 films were also irradiated at ENEA-National Institute of Ionizing Radiation Metrology with gamma radiation produced by a $^{60}\text{Co}$ source characterized by an absorbed dose to water rate of 0.26 Gy/min as measured by a calibrated Farmer type ionization chamber. EBT3 film calibration curves were determined by means of a set of 40 film pieces irradiated to various doses ranging from 0.5 Gy to 30 Gy absorbed dose to water. An EPSON Expression 11000XL color scanner in transmission mode was used for film analysis. Scanner response stability, intrafilm uniformity, and interfilm reproducibility were verified. Optical absorption spectra measurements were performed on unirradiated and irradiated EBT3 films to choose the most sensitive color channel to the dose range used.

Results: EBT3 GafChromic films show an under response up to about 33% for low-energy protons with respect to $^{60}\text{Co}$ gamma radiation, which is consistent with the linear energy transfer dependence already observed with higher energy protons, and a negligible dose-rate dependence in the 2–40 Gy/min range. Short- and long-term scanner stabilities were 0.5% and 1.5%, respectively; film uniformity and reproducibility were better than 0.5%.

Conclusions: The main purpose of this study was to implement EBT3 dosimetry in the proton low-energy radiobiology line of the TOP-IMPLART accelerator, having a maximum energy of 7 MeV.
Low-energy proton and $^{60}$Co calibrated sources were used to investigate the behavior of film response vs to be written in italicum dose. The calibration in 5 MeV protons is currently used for dose assessment in the radiobiological experiments at the TOP-IMPLART accelerator carried out at that energy value. © 2015 American Association of Physicists in Medicine. [http://dx.doi.org/10.1118/1.4926558]

Key words: EBT3 film dosimetry, proton dosimetry, low-energy protons

1. INTRODUCTION

The use of radiochromic film (RCF) dosimetry is widely consolidated for applications in photon, electron, and proton beams. It offers several advantages, in particular, 2D measurements of dose distributions with high spatial resolution, no postirradiation processing required, and low daylight sensitivity. In addition, it shows small linear energy transfer (LET) and energy dependence over a wide range of beam energies used in radiation therapy.

GAFCHROMIC® EBT3 films, recently commercialized by International Specialty Products (ISP, Wayne, NJ), are being widely used because of their good characteristics and improvements with respect to the previous model EBT2. Indeed, several studies have shown that particular attention is required when using EBT2 film, because of uncertainties regarding the influence of scanning orientation, film development time, and film uniformity. Conversely, EBT3 is more robust and easier to handle than EBT2 films. In EBT3 films, optical density changes stabilize rapidly (2-h waiting-time window), and dose–response uniformity is good (within 1.5%). Their symmetric layer configuration allows the user to eliminate side orientation dependence, and the presence of microscopic silica particles embedded into the polyester substrate prevents the formation of Newton’s rings in images obtained using a flatbed scanner.

Some studies have investigated the behavior of EBT3 films in photon, electron, and proton beams, mostly comparing EBT2 and EBT3 performances. In particular, EBT3 shows the same dosimetric response to photon and proton beams as its precursor, i.e., no dependence on radiation type, except for protons in the proximity of the Bragg peak. Recently, Reinhardt et al. suggest that care should be taken when using proton beams because of the considerable under-response of the film, which affects dose measurement accuracy. The investigation was conducted in a 200 MeV actively scanned clinical proton beam, with multiple film pieces placed perpendicular to the beam direction, at different depths inside a water phantom. An under-response of the film up to 5%, as compared to an ionization chamber, was found for energies below 40 MeV, and up to 20% close to the Bragg peak, corresponding to a very low residual energy of 4 MeV. The under-response has been mainly attributed to a quenching effect that occurs with higher LET along an incident particle track. However, as underlined by the same authors, under-response magnitude is related to energy spread and LET at a certain depth and varies with the energy of the incident proton beam. Therefore, attention has to be paid to compare energy quenching when different initial beam energies are involved. Devic et al. reported tests on EBT3 film in a 26.5 MeV proton beam focusing on the possible proton activation processes due to the very high dose rate expected by a cyclotron but did not report data on LET dependence of EBT3 films. Further studies with radiochromic films were done at 26.5 MeV proton energy, but using a different RCF model. No studies have been conducted at lower proton energy.

In the present study, we investigated the behavior of EBT3 in a 5 MeV energy proton beam. The work has been done in the framework of the Oncological Therapy with Protons (TOP)-Intensity Modulated Proton Linear Accelerator for RadioTherapy (IMPLART) Project launched by the Italian National Agency for New Technologies, Energy and Sustainable Economic Development, ENEA-Frascati, in collaboration with Istituto Superiore di Sanità, ISS, and Regina Elena National Cancer Institute, IFO.

The aim of the project is to build a proton therapy center based on an actively scanned proton beam produced by a pulsed (4 μs, 100 Hz) 3 GHz linear accelerator up to the final energy of 230 MeV. The segment up to 150 MeV is currently under construction at ENEA-Frascati which was chosen as the test site before transferring the machine to IFO. Protons are generated by a 3–7 MeV injector followed by a low-energy beam transport (LEBT) line matching the beam to the following accelerating modules. A deflecting magnet placed in the middle of the LEBT delivers the proton beam to a vertical beam line devoted to in vitro radiobiology experiments for the radiobiological characterization of the proton beam.

The vertical arrangement is particularly suitable for irradiating both cell monolayers and cells growing in suspension culture. Preliminary experimental work involves the measurement of cell survival and micronuclei, and chromosome aberrations in cells versus dose requiring an accurate determination of the dose. Among the available dosimetric methods, EBT3 GafChromic film dosimetry was considered the most convenient one in order to provide an accurate estimate of absorbed dose for dose levels involved in radiobiological experiments. This study is thus preparatory to the use of this system for the dosimetry of low-energy beams in that accelerator.

The present work presents the response vs to be written in italicum dose to tissue substitute (MS20), the material usually chosen as reference in radiobiological studies of EBT3 in proton beam energy of 5 MeV in the 0.5 Gy to 30 Gy dose range. In addition, the comparison of the response vs dose to water obtained in 5 MeV proton beam and $^{60}$Co gamma rays in the same dose range is also shown. Irradiation with the proton beam was performed at the Radiobiology irradiation facility of the 7 MV Van de Graaff CN accelerator at the INFN-Laboratori Nazionali di Legnaro-Padova (INFN-LNL), Italy; $^{60}$Co irradiation was performed at the National Institute of Ionizing Radiation Metrology, ENEA—INMRI, Rome, Italy.
2. MATERIALS AND METHODS

2.A. Dosimetry system, radiation sources, and dose measurements

The EBT3 GafChromic films used in this study are from the same lot No. A05021302. They were purchased in boxes containing 25 sheets. Each sheet is 203.2 × 254.0 mm² with a 0.028 mm thick active layer (sandwiched between two 0.125 mm thick layers of polyester). EBT3 film was considered suitable for our study, because film structure and dimensions allow 5 MeV protons to be completely transmitted through the active layer, as evaluated by the stopping and range of ions in matter (SRIM) code calculations (Fig. 1).

For our experiments, we used a special microcutter for printed circuits (Circuit Board Plotter LPKF Protomat C60) and an ad hoc steel mold to cut film pieces from a single sheet; a small line was drawn on each piece to keep track of the orientation with respect to the original sheet, paying attention not to flake off the sandwich structure of films. The film pieces were sized for the specific sample holders used for the two beam qualities: a 30 × 30 mm² square and a 13 mm diameter disk for exposure to gamma and to protons, respectively. We used an EPSON Expression 11000XL/PRO color scanner in transmission mode to measure the films.

For EBT3 readout, film pieces were placed, likewise oriented, at the center of the scanner bed, using a cardboard template to ensure film placement reproducibility. Films were scanned in the 48-bit color mode, with a spatial resolution of 200 dpi corresponding to a pixel size of 0.13 mm. The digital images thus obtained were saved in uncompressed tagged image file format (TIFF) and analyzed with the software ImageJ v1.46r (National Institutes of Health, Bethesda, MD) by sampling a predefined 1 × 1 cm region of interest (ROI) centered on the film image.

The described procedure was used for the readout of unexposed and exposed films.

As a first step, optical absorption measurements of unirradiated and irradiated EBT3 films were performed by using a double-beam and double-monochromator Perkin-Elmer Lambda 950 spectrophotometer at the Solid State Laboratory (UTAPRAD-MNF) of ENEA C.R. Frascati. The absorption spectra were collected in the 400–700 nm spectral range with a wavelength resolution of 1 nm. Each sample was fixed to a metal mask with a circular hole of 3 mm in diameter, mounted by means of special supports in the sample compartment, along the path of the measurement beam. As expected, the exposed films showed an increased absorption value at 636 nm in the investigated dose range.

For the measurements of this work, film uniformity and film-to-film reproducibility, short-, and long-term stabilities of the scanner were evaluated.

Film uniformity, as evaluated over 40 measurements in different positions of the same unirradiated sheet, and batch reproducibility, as measured on five sheets, were both within 0.5%. Short-term stability of the scanner, evaluated as the standard deviation of ten consecutive measurements of the same unexposed film piece, was better than 0.5%. Similarly, long-term stability calculated as the standard deviation of the mean value of four measurements of the same film piece, carried out once a week for 1 month, was within 1.5%.

The scanner response was converted to net optical density (netOD), defined as

$$\text{netOD} = \log_{10}(I_{\text{unexp} \rightarrow \text{bckg}} - I_{\text{exp} \rightarrow \text{bckg}}),$$

where $I_{\text{unexp}}$, $I_{\text{exp}}$, and $I_{\text{bckg}}$ are the red channel transmitted-intensities measured for unexposed films, exposed films, and zero light transmitted, respectively. The computed overall uncertainty on netOD is

$$\sigma_{\text{netOD}} = \frac{1}{\ln(10)} \sqrt{\frac{\sigma_{\text{unexp}}^2 + \sigma_{\text{bckg}}^2}{(I_{\text{unexp} \rightarrow \text{bckg}})^2} + \frac{\sigma_{\text{exp}}^2 + \sigma_{\text{bckg}}^2}{(I_{\text{exp} \rightarrow \text{bckg}})^2}},$$

where $\sigma_{\text{unexp}}$, $\sigma_{\text{exp}}$, and $\sigma_{\text{bckg}}$ are the uncertainties of the measured $I_{\text{unexp}}$, $I_{\text{exp}}$, and $I_{\text{bckg}}$.

We verified that the signal stability of the film was reached after about 8 h (with variations of film response lower than 0.2%), but for practical needs all the measurements reported in this work were performed 24 h after irradiation. The netOD was derived from the most sensitive red channel.

2.B. Proton irradiation

The proton irradiations were done at the Radiobiology irradiation facility of the INFN-LNL 7 MV Van de Graaff CN accelerator. The facility, the beam dosimetry, and the irradiation modalities have been described in detail in Belli et al. Briefly, the proton beam passes through two diffusing gold foils (2.2 mg/cm² thick, each), allowing the beam to broaden and become homogeneous on a circular surface ($\Phi = 13$ mm), where samples to be irradiated are normally positioned, and it is extracted in air through a bialuminized Mylar window (10 µm thick).

![Fig. 1. SRIM code outputs: energy loss in EBT3 (125 µm inert polyester layer + 28 µm active layer + EBT3 125 µm inert polyester layer) for 5 MeV protons. Simulated compositions of the layers (from ISP manual) are polyester = H 36.4%, C 45.5%, O 18.2%; active layer = H 56.8%, Li 0.6%; C 27.6%, O 13.3%, Al 1.6%.](image)
The EBT3 films were mounted at the bottom of a stainless steel Petri dish especially designed to fit the ion-beam geometry in air and to host cell cultures in sterile and wet conditions. The Petri dishes were placed in a rotating multisample holder (Fig. 2) which was remotely controlled during the irradiation experiment. After having traversed an air gap of 1 cm and the Mylar foil used as the base of the stainless steel Petri dish, the beam impinged on the EBT3 film (configuration A, Fig. 3). Protons fluence and energy at the EBT3 surface entrance were measured by a silicon surface barrier detector (SSBD) in air, located in the same position as the EBT3 films, and calibrated in energy with an Am-Cm-Pu alpha source. The ion-beam was monitored online during sample irradiations by means of two SSBDs, located in vacuum, along the beam line.

The setup sketched in Fig. 3(A) was normally used for EBT3 measurements. In order to investigate the beam quality dependence of the film response, two Mylar foils were added to reduce the proton energy, as shown in configuration B of Fig. 3.

The energy of the protons at the EBT3 entrance was 5 MeV. The absorbed dose at the EBT3 entrance was determined from the mean particle fluence and mean LET value calculated at the film surface according to the following relationship:

\[
\text{Dose (Gy)} = \left(1.6 \times 10^{-10} \times \text{Fluence} \left(\frac{1}{\text{cm}^2}\right) \times \text{LET} \left(\frac{\text{MeV cm}^2}{\text{g}}\right)\right). \tag{3}
\]

The mean LET value at EBT3 entrance was 77 MeV cm²/g as calculated from the mean energy value at the EBT3 film entrance considering the proton stopping power in MS20 from the ICRU 49 tables.

Ten dose values (0.5, 1, 3, 3.5, 4.5, 5, 8, 10, 20, and 30 Gy) and two dose rates (2.1 and 40 Gy/min) were used in this experiment. The uncertainty in the delivered dose was 5%. Two films were irradiated for each experimental condition.

### 2.C. ⁶⁰Co irradiation

The ⁶⁰Co gamma ray irradiations were done at ENEA-INMRI. The experimental setup is shown in Fig. 4. Films were placed in a 30×30×30 cm³ PMMA slab phantom (density = 1.18 g cm⁻³).

Each film piece was inserted between two PMMA slabs. The slabs were then aligned and clenched together to minimize the effect of air gaps in the phantom. The dose delivered by the beam was measured using a FARMER NE2571 (NE Technology Limited, Berkshire RG7 5PR, England) ionization chamber connected to a Keithley 6512 electrometer. The ionization chamber was previously calibrated against the absorbed-dose-to-water Italian Primary Standard. The
3. RESULTS AND DISCUSSION

3.A. Calibration curves for $^{60}$Co and protons

The calibration curves, dose vs netOD, for $^{60}$Co gamma rays and 5 MeV protons were obtained by irradiating EBT3 films with the two beam qualities in the same 0.5–30 Gy dose range in the experimental conditions described above. The experimental data were fitted by the following function:

$$D = a \times \text{netOD} + b \times \text{netOD}^n.$$

Figures 5(a) and 5(b) show measured data and fitting curves for $^{60}$Co gamma rays and for 5 MeV protons, respectively. The uncertainty in netOD obtained from Eq. (2) was on average 1% for photons and 2% for protons. Dose uncertainty was 1% for gamma rays and 5% for protons.

The best fit was obtained in both cases with $n = 3$ in the polynomial expression of Eq. (4), but using different values for the coefficients $a$ and $b$ as reported in Table I. The confidence limits of parameter values corresponding to a confidence level of 95% are also shown in parentheses.

According to Ref. 14, we calculated the combined uncertainty in dose determination as the quadratic sum of the uncertainty of fitting parameters plus the experimental uncertainty, and obtained a value between 8% and 3% for photons, and between 9% and 7% for protons.

Calibration curves for $^{60}$Co $\gamma$-rays and 5 MeV protons are compared in Fig. 6. The calibration curve for protons in MS20 material shown in Fig. 5(b) has been multiplied by a scaling factor $\text{LET}_{\text{water}}/\text{LET}_{\text{MS20}} = 106.3/77$ where $\text{LET}_{\text{MS20}}$ is the LET value in MS20 at 5 MeV, and LET$_{\text{water}}$ is the LET in water at 3.41 MeV, the energy at the middle of the active layer (see Fig. 1). In this way, we account for the water for both radiation types, and the energy degradation of protons in polyester and in the active layer. The curves show that the same dose corresponds to a lower net optical density for protons (vice versa for the transmitted-intensity), namely, 0.12 instead of 0.18 at 2 Gy, 0.41 instead of 0.54 at 10 Gy, 0.71 instead of 0.9 at 30 Gy: this corresponds to a lower darkening level for the EBT3 irradiated with protons of about 33%, 24%, and 21% at 2, 10, and 30 Gy, respectively.

To quantify the quenching effect, we applied the concept of relative efficiency (RE) of EBT3 according to the definition proposed by Martisikova and Jakel. In this work, RE expresses the ratio of doses to water of protons (3.6 MeV in

![Fig. 5. Calibration curves dose vs netOD of the EBT3 films. (a) Dose to water for $^{60}$Co gamma rays; (b) dose to MS20 for 5 MeV protons with 2.1 Gy/min dose rate.](image)

![Fig. 6. Comparison of the EBT3 calibration curves obtained with $^{60}$Co $\gamma$ rays (dashed line) and protons (solid line).](image)

<table>
<thead>
<tr>
<th>Parameters in Eq. (4)</th>
<th>60Co (dose to water)</th>
<th>5 MeV protons (dose to MS20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$a$ (95% confidence limits)</td>
<td>1025 (861, 1188)</td>
<td>1163 (998, 1329)</td>
</tr>
<tr>
<td>$b$ (95% confidence limits)</td>
<td>2852 (2541, 3163)</td>
<td>3744 (3373, 4116)</td>
</tr>
</tbody>
</table>
energy at the film active layer entrance) and photons (\(^{60}\text{Co}\))
needed to produce the same netOD in the EBT3 films. A RE
of about 0.6 at a dose of about 2 Gy was obtained. Our results
are consistent with the data reported in the literature, regarding
the under-response observed by other authors.\(^{12,26,28,29}\) In our
case, this behavior is enhanced because 5 MeV proton energy
is close to the minimum energy of the protons that can cross
the EBT3 film active layer (i.e., about 3.35 MeV, as evaluated
by SRIM code) which is almost the limit for the proper use
of EBT3 films. Additionally, the use of a pristine beam results in
a smaller energy spread at the Bragg peak and, consequently,
the under-response observed by other authors.\(^{12,26,28,29}\)

Moreover, the mean energy and the mean LET of the pro-
ton after the first Mylar foil and at the middle of the sensitive
layer in the A and B configurations, evaluated with SRIM
code, are reported in Table IV, where the uncertainties are
the standard deviations of the energy and LET distributions.

In the experimental condition B, a fluence \(F_B = 6.49 \times 10^6\)
protons/cm\(^2\) was delivered, corresponding to a dose of 8 Gy
MS20 evaluated after the first Mylar foil, and to a dose of \((26 \pm 2)\) Gy
at the middle of the sensitive layer, calculated using Eq. (3)
(Table IV). As a first approximation, the dose distribution in
the thickness of the sensitive layer can be considered linear.
Therefore, the dose value at the middle of the layer represents
the absorbed dose to the entire sensitive layer. The measured netOD
was 0.567 ± 0.003 (Table IV).

The correct comparison of this netOD value with that ob-
tained in configuration A must be done at the same dose to the
sensitive layer, i.e. \((26 \pm 2)\) Gy.

In the case of configuration A, this dose to the EBT3
sensitive layer corresponds to a fluence \(F_A = (1.48 \pm 0.11) \times 10^6\)
protons/cm\(^2\) [using Eq. (3)]. For this fluence, the dose
to MS20 at the EBT3 entrance in configuration A, calcu-
lated using Eq. (3), is 18.2 ± 1.3 Gy (Table IV). If no beam
quality dependence is expected for EBT3 response, this dose,
in configuration A, would produce the same netOD in the
sensitive layer as that obtained in configuration B, i.e., 0.567.
The EBT3 netOD value corresponding to a dose of 18.2 Gy
in configuration A was calculated from the calibration curve

### Table II. EBT3 film response (netOD) to 5 MeV proton beam with low dose rate, 2.1 Gy/min, and high dose rate, 40 Gy/min.

<table>
<thead>
<tr>
<th>Dose rate (Gy/min)</th>
<th>2.1</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Gy</td>
<td>0.468 ± 0.024</td>
<td>0.467 ± 0.024</td>
</tr>
<tr>
<td>20 Gy</td>
<td>0.673 ± 0.032</td>
<td>0.666 ± 0.033</td>
</tr>
</tbody>
</table>

### Table III. Computed average energy and FWHM of the energy spectra entering and exiting the EBT3 active layer in the A and B configurations.

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Average energy (MeV)</th>
<th>FWHM (MeV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.584</td>
<td>0.100</td>
</tr>
<tr>
<td>B</td>
<td>2.226</td>
<td>0.111</td>
</tr>
</tbody>
</table>

### Table IV. Average values of energy, LET, fluence, and dose evaluated after the first Mylar foil and at the middle of the sensitive layer in the A and B configurations. The uncertainties are the standard deviations of the distributions.

<table>
<thead>
<tr>
<th></th>
<th>Configuration A</th>
<th></th>
<th>Configuration B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After Mylar foil</td>
<td>Middle of sensitive layer</td>
<td>After Mylar foil</td>
<td>Middle of sensitive layer</td>
</tr>
<tr>
<td>(E) (MeV)</td>
<td>5</td>
<td>3.406 ± 0.055</td>
<td>5</td>
<td>1.095 ± 0.120</td>
</tr>
<tr>
<td>LET (MeV cm(^2)/g)</td>
<td>77</td>
<td>131 ± 3</td>
<td>77</td>
<td>299 ± 21</td>
</tr>
<tr>
<td>(F) (cm(^{-2}))</td>
<td>((1.48 ± 0.11) \times 10^6)</td>
<td>((1.48 ± 0.11) \times 10^9)</td>
<td>(6.49 \times 10^6)</td>
<td>(6.49 \times 10^6)</td>
</tr>
<tr>
<td>(D) (Gy)</td>
<td>18.2 ± 1.3 (to MS20)</td>
<td>26 ± 2 (to sensitive layer)</td>
<td>8 (to MS20)</td>
<td>26 ± 2 (to sensitive layer)</td>
</tr>
<tr>
<td>netOD</td>
<td>0.656 ± 0.025</td>
<td></td>
<td>0.567 ± 0.003</td>
<td></td>
</tr>
</tbody>
</table>

Medical Physics, Vol. 42, No. 8, August 2015
function [see Fig. 5(b)] as 0.656 ± 0.025 (Table IV). The difference between the two netOD values, obtained for configurations B and A, is statistically significant (r-Student test, \( P < 0.05 \)). The ratio between these two netOD values was about 1.16, a result in line with the data reported by other authors,\(^{36}\) indicating a beam quality dependence of EBT3 film at low energy (lower than 15 MeV). Specifically, EBT3 underestimates the dose with decreasing proton energy. No direct comparison between our data and those reported in the literature was possible because they were obtained using different initial proton beam energies.

4. CONCLUSIONS

The main purpose of this study was to implement EBT3 dosimetry in the TOP-IMPLART proton accelerator, specifically, in the proton low-energy radiobiology line devoted to cell irradiation which has a maximum energy of 7 MeV. We used calibrated sources to determine the behavior of the netOD film response vs dose in 5 MeV protons and ⁶⁰Co photons in the 0.5–30 Gy dose range. The maximum change in optical density between proton and photon calibration curves for EBT3 films was about 33% for a dose value of 2 Gy, corresponding to a RE value of 0.6. Results about dose rate and LET dependence in low-energy protons confirm a negligible dose-rate dependence of response in the 2–40 Gy/min range, and a LET dependence which, in our case, was of about 16% between protons of about 3.6 and 1.5 MeV at the entrance of the EBT3 active layer. These aspects will be the object of further investigations.

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Medical Physics, Vol. 42, No. 8, August 2015