A sensitivity study of micro-TLDs for *in vivo* dosimetry of radioimmunotherapy

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The sensitivity and precision of teflon-imbedded $CaSO_4$:Dy microthermoluminescent dosimeters (micro-TLDs) were determined. The micro-TLDs were sectioned from miniature TLDs (200 μm$\times$400 μm$\times$5 mm) that were fabricated using standard techniques. In order to measure absorbed dose, the miniature TLDs can be implanted directly into tissues (e.g., tumor xenografts) that have received injections of radiolabeled monoclonal antibodies. Micro-TLDs recovered from tissue sections cut with a microtome can be read out to determine local absorbed dose. The precision of dose estimation was quantified for uniformly irradiated 32-, 96-, and 192-μm TLD chips; coefficients of variation ranged from 22% to 41%, depending on chip size. The coefficients of variation were reduced to less than 12% using individual relative sensitivity factors for each micro-TLD. The spatial resolution of the micro-TLDs was studied by placing miniature TLDs across the sharp penumbral region of a linear accelerator x-ray field. TLDs were sectioned into 32-μm chips which were read out to determine the relative absorbed dose. The sharpness of the penumbra was readily quantified by the micro-TLDs.

Key words: microthermoluminescent dosimeters, *in vivo* dosimetry, radiolabeled antibodies, dosimetry, radioimmunotherapy

I. INTRODUCTION

Radiolabeled monoclonal antibodies (MoAbs) are being investigated as an alternative treatment modality against cancer because of their potential ability to deliver highly localized dose to targeted tumor cells.[1-3] However, uptake of radiolabeled MoAb and subsequent dose deposition in tissues may not be homogeneous.[4] Therefore, it is important to make quantitative measurements of the absorbed dose to tumor and normal tissue in order to establish criteria for radiolabeled MoAb treatments.

The established techniques for doing *in vivo* dosimetry include average dose calculations using time-dependent biodistribution data with the MIRD technique,[5] autoradiography,[6] and TLD implantation.[7] Each method has its merits and limitations. Biodistribution studies require a large number of animals and are very labor intensive. Therefore, tissues are counted at only a finite number of time points that may result in either an overestimation or underestimation of cumulated activity due to under sampling. MIRD calculations based on biodistribution data ignore the tissue-tumor contribution to dose from penetrating gamma rays and are based on the incorrect assumption that the radiolabeled MoAb and dose deposition are distributed homogeneously in tumor.[3,5] Autoradiography gives a highly resolved, detailed picture of heterogeneous MoAb deposition in tumor, but provides only a temporal snapshot of the dynamic process of activity deposition in only a thin section of the tumor. *In vivo* TLDs, on the other hand, provide a measurement of the integrated absorbed dose at the position of the TLD. However, the absorbed dose is averaged over a heterogeneous activity distribution because of the TLD dimensions. To overcome this limitation, Griffith *et al.* have pioneered a technique[4] of using micro-TLDs to measure absorbed doses in small, localized regions. Those measurements can also be compared with doses determined from autoradiography activity distributions.[8]

In order to use the micro-TLDs for *in vivo* dosimetry, it is necessary to quantify the precision with which they can be used to estimate dose. This paper presents data from precision and sensitivity measurements of micro-TLDs under controlled conditions using external beam irradiation, a best case scenario. Measurements were also made to study the effects of *in vivo* irradiation.

II. MATERIALS AND METHODS

Micro-TLDs were manufactured according to the methods described by Wessels and Griffith.[7] $CaSO_4$:Dy impregnated, 400-μm-thick teflon disks[9] were cut into 5-mm-long strips. The strips were imbedded in paraffin and cut with a microtome every 200 μm to yield miniature TLD rods measuring 5000$\times$200$\times$400 μm^3. $CaSO_4$:Dy was selected as the TLD material because of its superior sensitivity and fading characteristics.[10] The miniature TLD rods were cleaned by carefully scraping off excess paraffin. The TLDs were annealed at 270 °C for 10 min and irradiated to 10 Gy under full buildup conditions in a ^{60}Co beam. The miniature TLD rods were mounted at -21 °C in O.C.T. compound, a water soluble compound[11] used for imbedding and mounting frozen tissues for microtome sectioning. They were cut into transverse sections along their length to produce many 32-, 96-, or 192-μm-thick micro-TLD chips (each with a cross-sectional

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area of $200 \times 400\ \mu m^2$). The micro-TLDs were picked from the imbedding compound sections under a microscope using jewelers tweezers and the residual compound was removed by soaking the micro-TLD chips in a drop of distilled water for several minutes. This method of imbedding and recovery best preserved the integrity of the micro-TLDs that were visually checked under a dissection microscope.

The individual micro-TLDs were positioned in the middle of a TLD reader[12] planchet that was heated to 125 °C and maintained at that temperature for 30 s in order to deplete low-energy traps. The temperature was monotonically raised to 275 °C in 15 s and maintained at that temperature for an additional 30 s in order to capture the entire signal under the high-temperature tail of the glow curve. The TLD signal was integrated over this latter 45-s time interval. There was no systematic difference in the readings from micro-TLDs of the same thickness cut from different miniature TLDs irradiated to the same dose. The readings were grouped together in order to obtain large sample sizes. Means (μ), standard deviations (σ), and coefficients of variation ($100 \cdot \sigma/\mu$) were computed for the distributions of readings for each group.

For some of the experiments, the micro-TLDs were read out as described above and then placed in an aluminum tray with individually marked wells. Those micro-TLDs were annealed at 270 °C for 10 min to remove residual signal ($< 1\%$ of reading from the first irradiation) and to reestablish the preirradiation condition of the TLDs. The TLDs received a second 10-Gy irradiation. Readings from the second irradiation were used to calculate a relative sensitivity factor SF_i for each micro-TLD:

$$SF_i = X/X_i,$$

where X_i was the individual second reading for the ith TLD and X was the average reading for each group of TLDs. SF_i was multiplied by the original reading of the ith micro-TLD to give a final corrected reading. This method of individually calibrating the TLDs is called the modified readout procedure.

Some miniature TLDs were injected into a tumor xenograft with a 20 gauge spinal needle. A miniature ($200 \times 400 \times 5000\ \mu m$) TLD is placed in the needle that is used to puncture the tumor and the TLD is pushed into the tumor with a plunger that fits inside the needle. The tumor and the TLD then receive approximately 10 Gy from a ^{60}Co beam. That dose was chosen as previous biodistribution studies[3] have shown that maximum tumor dose from injected radiolabeled MoAb is on the order of 10–20 Gy. After 4 days the tumor was removed and cut into 32-μm sections along the length of the implanted miniature TLD. The sections were placed on glass slides and allowed to air dry. The micro-TLDs were removed from the tissue sections using jeweler's tweezers, cleaned in deionized and distilled water, read out, and then individually calibrated using the modified readout procedure. The annealing time for the modified procedure was varied from 5–15 min to study the effects of TLD discoloration during the annealing process.

Some miniature TLDs were taped against the lower collimator of a linear accelerator[13] such that half of the TLD was

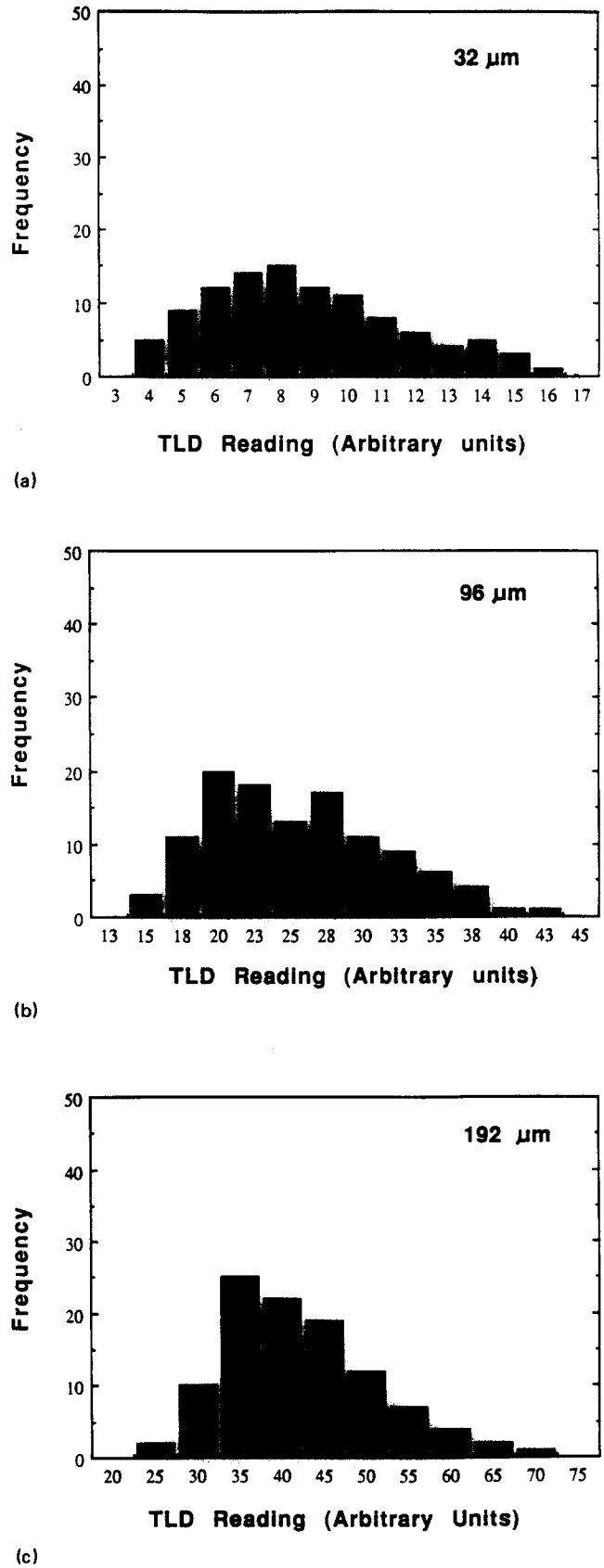

FIG. 1. Frequency histograms of uncorrected readings for uniformly irradiated (a) 32 μm, (b) 96 μm, and (c) 192-μm micro-TLDs using the standard readout procedure. The means and standard deviations are (a) 9.4 ± 3.0, (b) 26.9 ± 7.1, and (c) 44.3 ± 9.5. The coefficients of variation are 32%, 26%, and 22%, respectively. The bin size is approximately 10% of the mean for each distribution.

in the field and half was shielded by the collimator, thereby creating a very sharp dose gradient across the TLD. The in-field portion of the TLD was given a dose of approximately 10 Gy using 10-MV photons. Those TLDs were removed from the collimator, imbedded in O.C.T. compound and sectioned into 32-μm chips. Sensitivity factors were calculated for the micro-TLDs using the modified readout procedure. Radiographic film[14] was placed in the same location as the TLD on the collimator face and received an exposure of approximately 1 Gy. The film was scanned with a laser film densitometer[15] using a 100-μm spot size and a 100-μm step size to obtain an optical density profile that was subsequently compared to the micro-TLD readings.

III. RESULTS

The distributions of readings for the uniformly irradiated 32-, 96-, and 192-μm micro-TLDs are presented in Figs. 1(a)–(c). The readings were not corrected for individual sensitivity factors. The sample size for each micro-TLD thickness was approximately 100. The ratio of the mean reading of the 32-μm TLD distribution to the mean reading of the 96-μm distribution is 1:2.9 and the ratio for the 32-μm TLD to the 192-μm TLD mean reading is 1:4.7. These ratios scale approximately as the relative thickness of the micro-TLDs. The coefficients of variation (Table I) are unacceptably large for precise dosimetric measurements.

Using the modified procedure to apply individual relative sensitivity factors to scale the original test readings [Fig. 1(a)–(c)] dramatically improves the coefficients of variation to less than 12% for all sized micro-TLDs [Fig. 2(a)–(c)]. As expected, the means of the distributions using the modified procedure are nearly the same as the means using the standard procedure. The sample sizes are smaller by approximately 10% because of TLD loss due to the increased amount of handling that was necessary for the modified procedure.

Figure 3 shows the frequency distribution of readings from micro TLDs recovered from 32-μm sections of mouse tumor. The coefficient of variation is 41% using the standard readout procedure [Fig. 3(a)] which is larger than the coefficients for TLDs that were irradiated in phantom. However, when the readings are corrected for individual

TABLE I. Means, standard deviations (σ), and coefficients of variation (CV) for distributions of readings for uniformly irradiated micro-TLDs using the standard and modified readout procedures. The sample size for each TLD thickness is approximately 100.

TLD thickness (μm)	Readout procedure	Mean	(σ)	CV
32	standard	9.4	(3.0)	32%
32	modified	9.5	(1.0)	11%
96	standard	26.9	(7.1)	26%
96	modified	27.4	(3.1)	12%
192	standard	44.3	(9.5)	22%
192	modified	44.8	(5.1)	11%
32 (*in vivo*)	standard	6.8	(2.8)	41%
32 (*in vivo*)	modified	6.9	(0.7)	10%

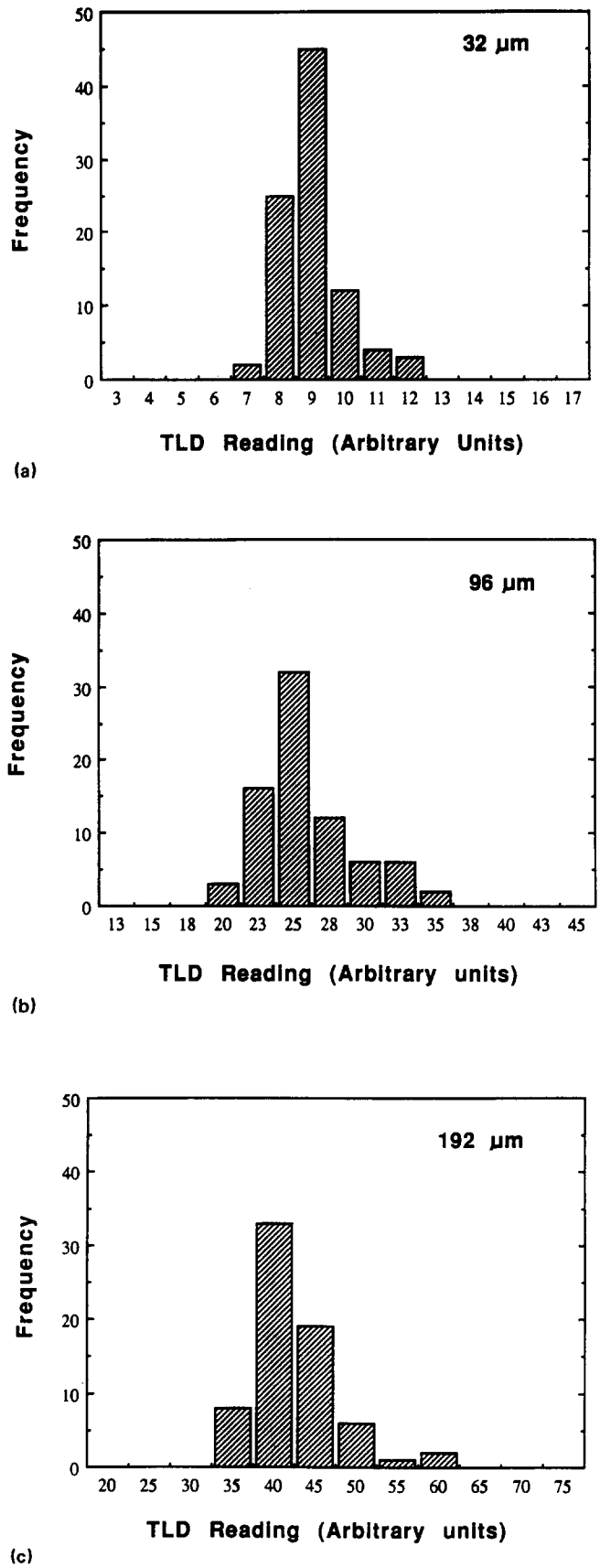

FIG. 2. Frequency histograms of readings for (a) 32 μm, (b) 96 μm, and (c) 192 μm TLDs using the modified readout procedure. The means and standard deviations are (a) 9.5 $\pm$ 1.0, (b) 27.4 $\pm$ 3.1, and (c) 44.8 $\pm$ 5.1. The coefficients of variation are 11%, 12%, and 11%, respectively. The bin size is approximately 10% of the mean for each distribution.

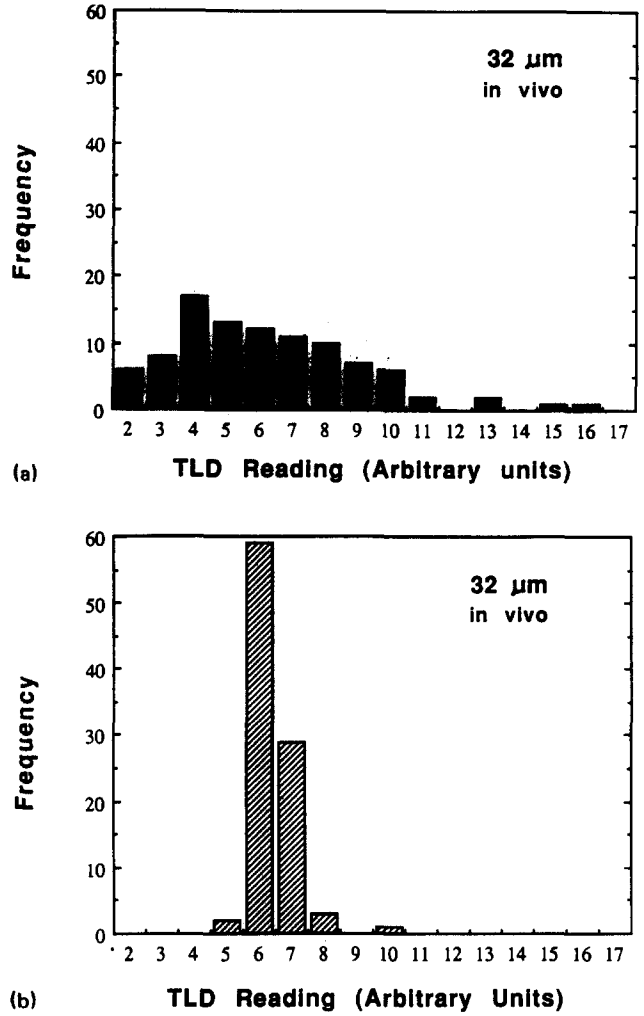

FIG. 3. Frequency histograms of (a) uncorrected and (b) corrected readings for uniformly irradiated, *in vivo* 32-μm TLDs. The means and standard deviations are (a) 6.8 ± 2.8 and (b) 6.9 ± 0.7. The coefficients of variation are 41% and 10%, respectively. The bin size is approximately 15% of the mean for each distribution.

sensitivity the coefficient of variation improves to 10% [Fig. 3(b)], which is consistent with the coefficients for the corrected readings of the TLDs irradiated in phantom.

Figure 4 shows overlaid plots of raw and corrected readings from 32-μm TLDs as a function of position along the miniature TLD and a normalized optical density (O.D.) profile from a film placed at the same location as the miniature TLD on the face of a collimator of a linear accelerator. The O.D. readings have been normalized to the corrected TLD readings for comparison. This experiment demonstrates the ability of the micro-TLDs to spatially resolve dose differences in a region of large dose gradient.

IV. DISCUSSION

The results presented in Figs. 1 and 2 show that, in our hands, it is essential to compute individual sensitivity factors for the micro-TLDs in order to obtain reasonably precise

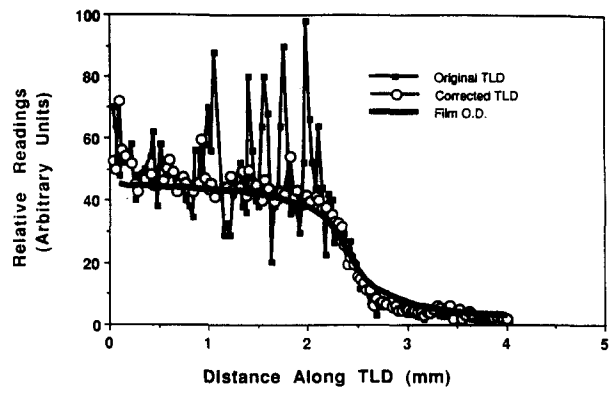

FIG. 4. Corrected and uncorrected micro-TLD readings from a miniature TLD placed across the penumbra of a 10-MV photon beam compared to a film placed at the same location. The film O.D. is normalized to the TLD readings.

dose values. The coefficient of variation using the standard readout procedure ranges from 32% for the 32-μm TLDs to 22% for the 192-μm TLDs; using the modified procedure, the coefficients of variation range from 11% to 12%. Using similar TLD material and fabrication methods, Griffith *et al.*[4] reported a coefficient of variation of 10% for 20-μm TLDs which is consistent with our value of 11% using the modified readout procedure.

The large coefficients of variation for the 32-μm TLDs using the standard readout procedure may be explained by variations in TLD thickness and the amount of $CaSO_4$:Dy crystal contained in each micro-TLD. The thickness of TLDs cut with a microtome was experimentally determined to have a standard deviation of ± 5%. The distribution of $CaSO_4$:Dy content per TLD also contributes to the overall standard deviation of the TLD readings. Although it is neither practical nor necessary to determine quantitatively the distribution, it can be qualitatively observed using a dissection microscope at 50X magnification. In addition to the inherent variability in $CaSO_4$:Dy content, examination under the dissection microscope revealed that approximately 5% of the TLDs were missing a substantial portion (> 10%) of their volume due to large $CaSO_4$:Dy crystals which had been pulled from the teflon matrix during the cutting and/or handling process. In order to minimize this problem, the microtome knife was routinely sharpened and inspected under a microscope. Moreover, the miniature TLDs were imbedded in frozen O.C.T. compound rather than another imbedding medium such as paraffin because O.C.T. compound best immobilized the TLD during cutting, facilitated micro-TLD extraction from microtome sections and minimized handling of the micro-TLDs.

Thicker 92- and 196-μm micro-TLDs were sectioned in an attempt to improve the coefficients of variation for the standard readout procedure. It was felt that the larger TLDs would be affected less by size variations due to the cutting procedure and that the distribution of $CaSO_4$:Dy crystals among the TLDs would have a smaller relative spread than for the thinner 32-μm TLDs. The coefficients of variation

were smaller than for the 32-μm TLDs, but were still too large for dosimetry measurements. Like the 32-μm TLDs, the thicker TLDs gave adequately precise readings only when using the modified readout procedure. However, it is preferable to use thinner tissue sections for autoradiogrpahy experiments. The tissue should be thick enough to provide sufficient activity for well-exposed autoradiographs, but it should be thin enough not to degrade the resolution.

Miniature TLDs were irradiated and sectioned *in vivo* in order to simulate actual conditions for radiolabeled MoAb experiments. We have observed previously that miniature TLDs which have been implanted in tissue may become discolored when heated during readout. The likely cause of this discoloration or "browning" is the carbonization of proteins that adhere to the exposed surfaces of the TLDs. Despite rigorous efforts to clean proteins from the TLDs using various proteases (proteins that cut amino acid chains at specific bond sites), the browning effect has persisted and varies from TLD to TLD and is often nonuniform over the surface of a single miniature TLD. It also has been observed that the teflon matrix becomes discolored when control TLDs are heated to high annealing temperatures, however, this discoloration is qualitatively much less than the "browning" effect observed for *in vivo* TLDs. A relatively low annealing temperature, 270 °C, and a short annealing time, 10 min, were chosen to minimize this effect.

The *in vivo* micro-TLDs in this study showed some signs of browning; the degree of browning appeared to be proportional to the length of annealing time. In one study, a group of 100 micro-TLDs had a coefficient of variation of 26% after the first reading. The TLDs were annealed for 15 min, irradiated and read out a second time. A large number of TLDs were noticeably browned after the annealing and the coefficient of variation of the corrected readings was only 25%. Clearly, the browning affected the TLD readings and degraded the results of the modified readout procedure compared to the results from the control measurements. A second study that used the same readout procedure was performed using a shorter 5-min annealing time. After the first reading, the coefficient of variation was 41%. Following the 5-min anneal, there was no discernible browning of the TLDs and the residual readings were less than 1% of the original readings. The coefficient of variation for the corrected readings was 10%, which is acceptable for dosimetry measurements and is consistent with the control measurements. The conclusion from these studies is that a 5-min anneal at 270 °C is sufficient to remove most of the residual signal ($<1\%$) while minimizing the amount of TLD browning and it is sufficient for adequately precise (coefficient of variation $<10\%$) dosimetry measurements.

All TLDs used in this study received a dose of 10 Gy; however, the mean reading for the *in vivo* measurements was 28% lower than the mean reading for the control micro-TLD measurements. The reason for this apparent discrepancy may be due to "*in vivo* signal fading." The amount of signal loss is proportional to the length of time the TLD remains *in vivo* and is postulated to be due to a combination of temperature effects and loss of TLD crystal via dissolution in the aqueous *in vivo* environment. This is a significant problem that cannot be ignored and requires further investigation.

By applying individual sensitivity factors, micro-TLDs can be used *in vivo* to determine doses with a precision of better than $\pm 12\%$. Moreover, the TLDs can be used to measure large dose gradients similar to what is encountered in the tumor of an animal that has been injected with radiolabeled MoAbs. Other issues such as *in vivo* signal fading and the effects of dose rate remain unexplored and need to be addressed so that micro-TLDs can be used in conjunction with other techniques to gain a more complete understanding of dose delivered to tumor using radiolabeled MoAbs.

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