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ELECTRON MICROSCOPY AND COMPUTED MICROTOMOGRAPHY STUDIES OF IN VIVO IMPLANTED MINI-TL DOSIMETERS

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The need for direct methods of measuring the absorbed dose in vivo increases for systemic radiation therapy, and in more sophisticated methodologies developed for radioimmunotherapy. One method suggested is the use of mini-thermoluminescent dosimeters (TLD). Recent reports indicate a marked loss of signal when the dosimeters are used in vivo. We investigated the exterior surface of the dosimeters with scanning electron microscopy and the interior dosimeter volume with computed microtomography. The results show that the dosimeters initially have crystals uniformly embedded in the teflon matrix, with some of them directly exposed to the environment. After incubation in gel, holes appear in the dosimeter matrix where the crystals should have been. The computed microtomographic images show that crystals remain in the interior of the matrix, producing the remaining signal. We conclude that these dosimeters should be very carefully handled, and for practical use of mini-TLDs in vivo the dosimeters should be calibrated in equivalent milieus. An alternative solution to the problem of decreased TL efficiency, would be to coat the dosimeters with a thin layer, of Teflon, or other suitable material.

For systemic radiation therapy (SRT), e.g., with radiolabeled monoclonal antibodies (MAbs) there is a greater need for methods of measuring absorbed doses in vivo. In radioimmunotherapy (RIT) much effort has been devoted to developing methods for calculating absorbed doses in tumors and radiation-sensitive normal tissues (1–9). However, these absorbed doses are only estimates, and the individual patient can, because of the actual biokinetics, receive absorbed doses very different from the estimated dose. Thus, for macroscopical dosimetry, methods for the direct measurements of the absorbed doses in vivo are highly desirable. One method suggested is the use of small thermoluminescent dosimeters (mini-TLDs). We have used Teflon-embedded $CaSO_4:Dy$ thermoluminescent dosimeters (10–13) measuring $0.2 \times 0.4 \times 5\ mm^3$.

None of the early studies with mini-TLDs mentioned the loss of signal. We tried to correlate mini-TLD measurements with calculations of absorbed doses in an animal experiment with ^{131}I labelled antibodies. Because we consistently found discrepancies between the results, we investigated the signal from the mini-TLDs when used in vivo.

We found that the signal from these dosimeters decreased markedly when they were implanted in vivo (14) and that this effect was strongly pH-dependent. One possible explanation of the loss of TL signal is that the TL-crystals dissolve in a liquid environment, creating 'holes' (voids) in the dosimeter matrix. Demidecki et al. (15) independently documented signal fading in very thin $CaSO_4$-Teflon discs. The question is whether these voids penetrate into the dosimeter and cause the disappearance of crystals also in the interior. Here we report studies of crystal loss from rod-shaped dosimeters. Scanning electron micro-

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scopy (SEM) was used to image the surface, and computerized microtomography (CMT) with synchrotron x-rays was used to image the interior of selected dosimeters.

Material and Methods

Mini-TL dosimeters were prepared from 0.4 mm thick $CsSO_4$: Dy loaded, 13 mm diameter Teflon discs. The discs were embedded in paraffin and sectioned with a microtome to a size of $0.4 \times 0.2 \times 5$ mm^3. The dosimeters were washed in xylene to remove the paraffin after sectioning.

To investigate the effect on loss of TL signal after implantation of the dosimeters in gel and tissue, irradiated mini-TLDs were implanted in solid gel (consisting of 4 g gelatin powder dissolved in 100 ml hot water) and fresh muscle tissue, and were left in darkness for up to 9 days. This was done at two different temperatures: 4°C and 22°C. The mini-TLDs were then removed washed in detergent and water, then rinsed in water and finally rinsed in distilled water three times.

To examine the surface, mini-TLDs were imaged with a scanning electron microscope (JEOL JSM T330) at the Unit of Electron Microscopy, Department of Animal Physiology, University of Lund. The microscope was operated at 15 kV at a magnification of ×150 and ×350.

The high resolution CMT system at the X26 Microprobe beamline of the National Synchroton Light Source at Brookhaven National Laboratory (16, 17) was used to non-destructively image transversal slices through the interior of the dosimeters. First generation scanning was done with a pencil beam resulting in 2 μm transverse resolution and 2 μm slice thickness. An unfiltered x-ray beam was used in the experiment because of technical constraints on the set-up available for this pilot study. The x-ray energy spectrum was therefore not optimized for the samples, but the detrimental effects of the high energy photons in the unfiltered synchrotron x-ray spectrum were reduced by using a relatively thin (1 mm) CaF_2 scintillator crystal as the detector.

Results

The result of readings from the incubation experiment of the mini-TLDs in gel and muscle tissue are given in Fig. 1. The curves represent the relative TL signal as a function of storage time in gel and muscle tissue at different temperatures.

Fig. 2a shows a SEM micrograph of a virgin mini-TLD rod. The dosimeter appears to have a smooth surface and straight edges. TL crystals are clearly visible on the surface of the dosimeter at both the low (Fig. 2a) and high magnification (Fig. 2b). It is obvious that these crystals are susceptible both to sectioning and handling with tweezers, and to chemical attack when immersed in fluids. A few voids, probably resulting from loss of crystals during manual handling of the dosimeters, can be seen in Fig 2a and b.

After storage of the dosimeters in gel, the surface changed dramatically (Fig. 3a and b). Many voids are distributed over the surface of the dosimeter, and some crater-like 'holes' can also be seen. At high magnification, a large proportion of the crystals on the surface are apparently missing.

Fig. 4a shows an attenuation tomogram of a virgin dosimeter, using a false color scale. The $CaSO_4$ crystals appear as bright yellow-reddish spots in the image, the

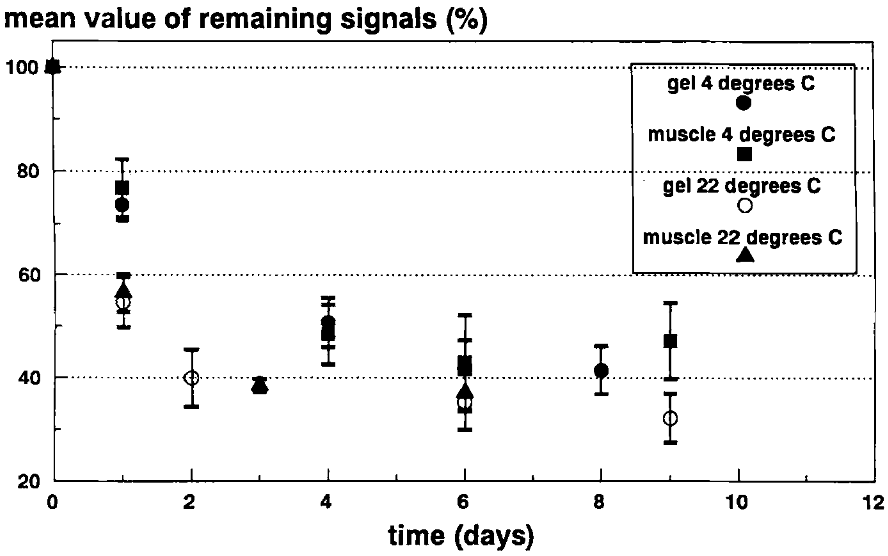

Fig. 1. Fading of the signal as a function of time from the mini-TLD after ^{60}Co-irradiation before implantation. Fading in gel and muscle tissue is almost the same, and is enhanced at higher temperatures.

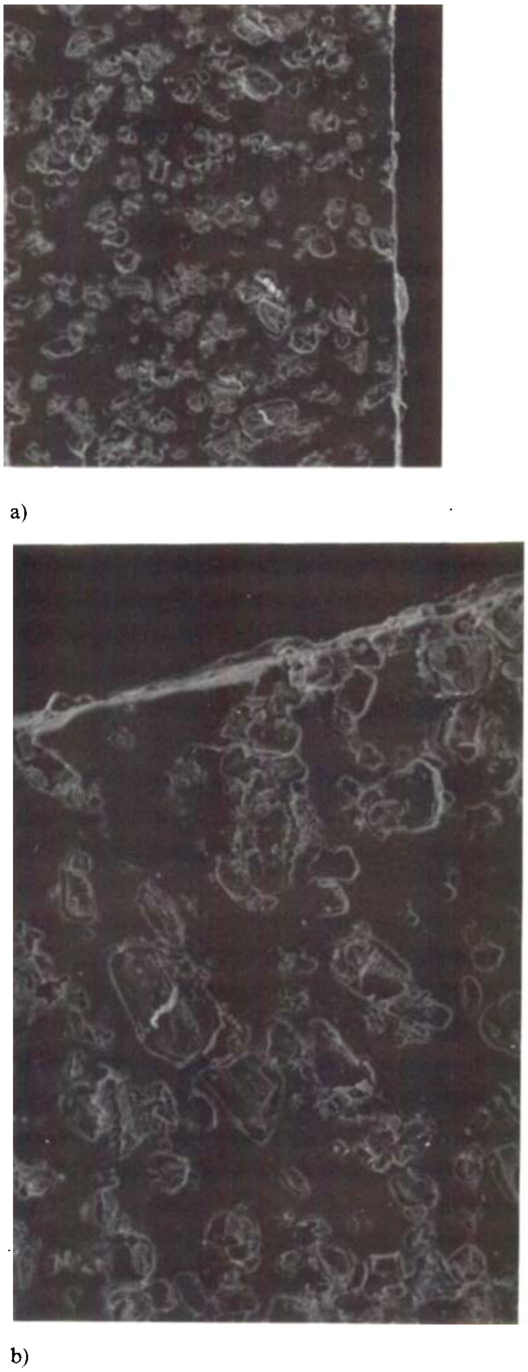

Fig. 2. SEM images of a virgin mini-TLD before implantation in gel. a) Magnification × 150 and b) magnification × 350. Note crystals close to the surface of the Teflon matrix.

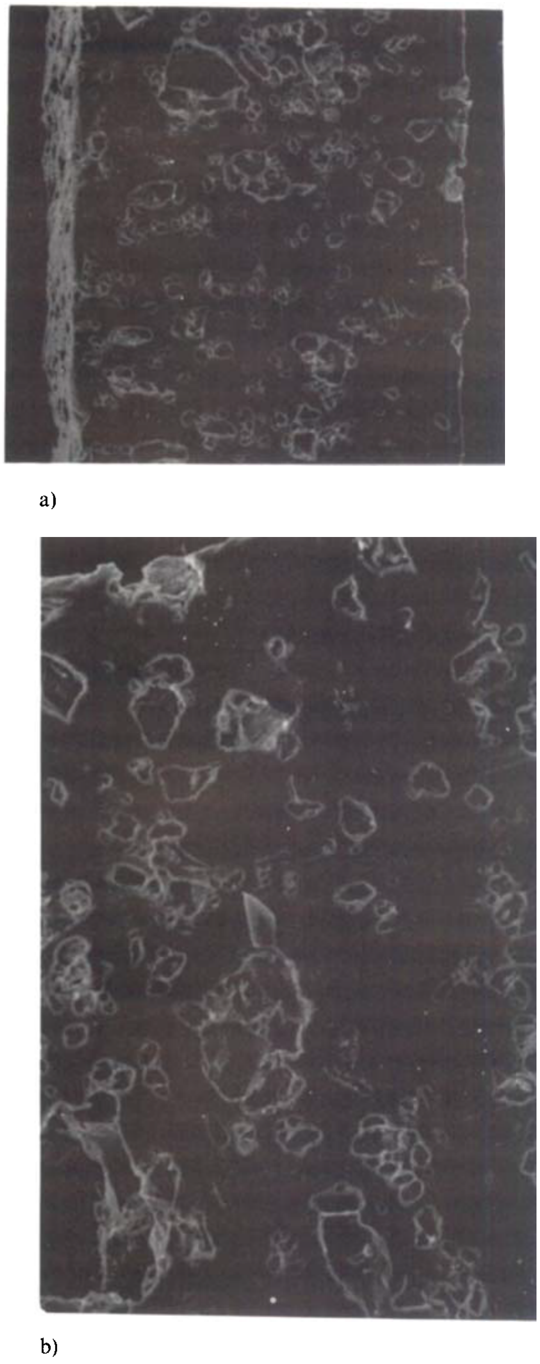

Fig. 3. SEM images of a mini-TLD 5 days after implantation in gel. a) Magnification × 150 and b) magnification × 350. Several crystals are missing, leaving voids in the Teflon matrix.

Telflon matrix apears blue and green, and air is black. The crystals are distributed randomly throughout the Teflon matrix, some extending out to the surface of the dosimeter. Fig. 4b is an attenuation tomogram of a dosimeter stored for 5 days in gel. A couple of large voids, probably the result of loss of relatively large crystals, can be seen close to the surface. However, many crystals are still present in the interior of the dosimeter even after implantation for as long as 5 days. Signal loss was about 70% after 9 days at 22°C, and the loss of signal increased with temperature. At each temperature, signal loss in gel and muscle tissue appeared to be the same.

Discussion

Both the SEM and CMT images show that the dosimeters initially have crystals embedded close to, or extending to, the surface of the dosimeters. The crystals are therefore clearly exposed to the environment in which the dosimeters are stored. After implantation in gel, voids appear at or close to the surface of the dosimeters, indicating loss of crystals. The micro-tomograms show that a considerable proportion of the crystals are still present in the interior of the dosimeters even after 5 days of implantation, an observation in agreement with the fact that a significant TL signal is still emitted from these dosimeters.

In our previous study (14), the mini-TLDs showed considerable individual variation in sensitivity after irradiation with equal absorbed doses (maximum variation about 40%) and had to be handled individually throughout all the experiments. This might be because crystals on the surface becomes loosened and drop off during cutting, washing in xylene and handling.

Previous signal loss experiments (14) showed that fading in air is slight in darkness, reaching about 10% after 9 days. No great difference could be seen at different temperatures. The experiments in gel and muscle (Fig. 1) showed a marked loss of signal after incubation. Signal loss was also strongly pH-dependent being about the same in liquid and in gel. The loss of signal was considerable for pHs below 6. The pH sensitivity of the mini-TLDs probably depends on the fact that hydrogen ions in the fluids and the gels dissolve the $CaSO_4$ crystals from the dosimeters to a different extent, depending on the concentration of hydrogen ions, i.e., the pH.

To conclude, this investigation showed clear evidence of crystal loss due to dissolution from the Teflon matrix when the dosimeters were imbedded in vivo. The images of both SEM and micro-CT showed loss of crystals from the dosimeter surface. The results are in accordance with our previous experiments, in which we found that the signal of the mini-TLDs was dependent on storage time in gel or muscle and on pH. To be able to use mini-TLDs in vivo these conditions must be taken into consideration.

It should be noted that the TL signal does not level off to a constant value, but continues to decrease throughout long storage periods. This indicates that dissolution of the crystals probably continues, and that pretreatment consisting of dissolving surface-connected crystals by immersion in a suitable fluid cannot be used as a remedy for signal decay during stroage. For such a method to be effective, the voids appearing in the dosimeters can not be interconnected with crystals in the interior. Computerized microtomography could be used to investigate whether such interconnectivity exists. An alternative solution to the problem of declining TL efficiency may be to coat the dosimeters with a thin layer of Teflon or some other suitable material.

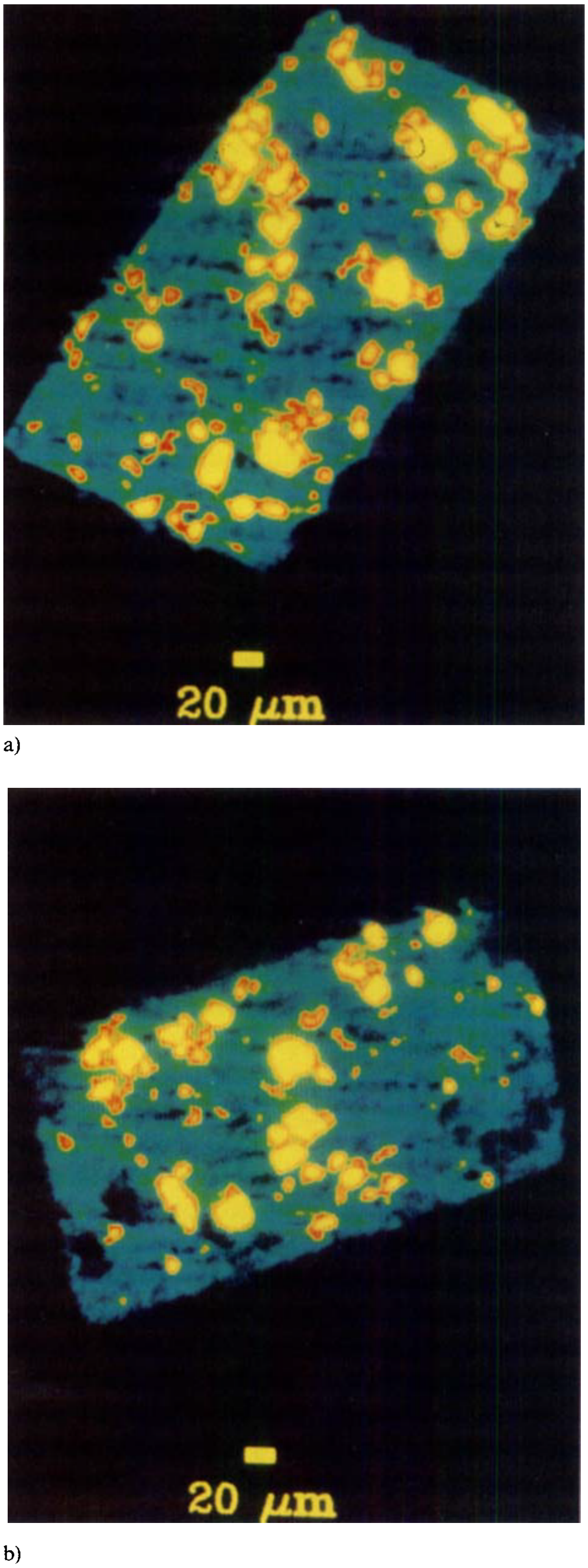

Fig. 4. Micro-CT images of a mini-TLD. Transverse sections of the TLD a) before treatment shows crystals throughout the Teflon. b) After 5 days of treatment 'holes' have appeared where crystals are missing close to the surface, whereas other crystals remain inside the dosimeter.

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