

BACKSCATTER RADIATION AT TISSUE–TITANIUM INTERFACES

Biological effects from diagnostic 65 kVp x-rays

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The induced secondary electrons from a metal surface by diagnostic x-rays are thought to contribute to cell damage near the tissue–metal boundaries of metal implants. Titanium implants are becoming increasingly more popular for tissue reconstructions and it is rather often desirable to take radiographs of the operated area. In this study we compared the biological effects of radiation on cultured mammalian test cells grown on titanium plates with the radiation effects on cells that were grown on plastic control plates. In order to study the acute radiation effects on cell growth it was necessary to work with rather high radiation doses (0.7–5 Gy). Photon energies, suitable for diagnostic radiography in odontology, 65 kV, were applied. We found that the cells grown on titanium plates were, in terms of the applied dose in the surrounding culture medium, more sensitive to the irradiations than the cells growing on plastic plates. The survival curve for the cells on titanium had a steeper slope, showed no shoulder in the low-dose region and looked like curves normally obtained for high LET radiation. It was not possible to resolve to what degree the titanium-dependent changes were due to an increased dose near the titanium surface or to a change in the radiobiological effectiveness. Although there was a significant decrease in cellular survival near the metal, postoperative intraoral radiography after titanium implantations need not be excluded. The maximal doses given in odontological x-ray examinations are less than 1 mGy and, if the results in this study are applied, the biological effects near the titanium implant will correspond to biological effects in soft tissue of doses less than 20 mGy which is lower than the doses that give acute effects. The risk of acute healing disturbances are significant only at much higher radiation doses.

Titanium and stainless steel are used as implants in maxillofacial reconstructions and titanium is also increasingly used for anchorage of tooth prostheses in odontology and for fixation of facial prostheses. The advantage of

using titanium is its ability to integrate with bone, resulting in a direct structural and functional connection between ordered, living bone and the surface of the load-carrying implant (osseointegration) (1).

Metal implants perturb radiation dose distributions due to the build-up of backscatter radiation in the entrance boundary zone between metal and tissue. The major contribution to this backscatter radiation is the release of secondary electrons from the metal surface. The absorbed dose in the boundary zone can, under certain conditions, increase by as much as 25–40 times the absorbed dose from the unperturbed radiation field (2). The enhancement factors increase with an increase in atomic number of the metal, making the effects to be more dramatic near stainless steel compared to titanium implants.

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Due to the expected dose increase, the radiation doses are generally kept as low as possible during diagnostic investigations. For example, in intraoral radiography the average absorbed dose has been greatly reduced during the two last decades (3) and in implant surgery it has been recommended that radiographic procedures should not be carried out during the healing period (4).

Experimental measurements, at distances up to several cell layers from metal interfaces using thermoluminescent LiF detectors (0.13 mm thick) combined with spacers consisting of successive layers of 5 μm mylar foils, have been published by Alm-Carlsson (2). A dose enhancement factor of about 40 was obtained with radiation of diagnostic quality (100 kV x-rays) applied to a Pb surface (2).

Stenström et al. (5) calculated both the volume of tissue exposed to secondary electrons from implanted metals and the increase of absorbed doses in this volume. They used a theoretical model for electron transport and found that the distance from the implant exposed to secondary electrons is limited to about 20 μm . At a dose to soft tissue of 1 mGy (65 kV x-ray photons, 2 mm Al filtration), after the penetration of 10 mm soft tissue and assuming that the bone tissue around the implant is composed of half trabecular and half cortical bone, the maximum dose to the bone marrow at a point on the implant surface was estimated to be about 27 mGy. This dose could be nearly doubled in the angle at the bottom of threads because of electron contribution from two directions.

Increased radiation-induced mucosal reactions due to therapeutic irradiations (e.g. 4–6 MV x-rays or ^{60}Co gamma radiation) have been reported adjacent to gold crowns, amalgam fillings (6) and mandibular reconstructions stabilised by metal tray implants (7). For the purpose of mandibular surgery using metallic implants, Tatcher et al. (8) measured the dose perturbation of mesh implants, finding clinically significant overdosage at the entrance side and a corresponding underdosage at the exit side. High energy therapeutic photon radiation generally results in lower dose enhancement factors than the low photon energies used in radiography.

An important question is whether there is a risk of cell injury in the tissue layer around the metal implants even after a low dose of diagnostic radiation. If so, radiography might disturb the tissue integration of, for example, titanium implants during the sensitive healing phase. The healing process is dependent on proliferating connective tissue and such tissue is known to be radiosensitive (9). In the absence of adequate knowledge, radiographic procedures have been discouraged immediately after implant operations (4). However, there are clinical situations where early postoperative radiographic procedures may be of great value, such as in cases of healing complications, reconstruction operations with additional implantations and in the case of a fracture during the operation or later in the healing period. Moreover, in many instances refer-

ence radiographs taken shortly after the operation are valuable for comparisons with later follow-up radiographs. It is therefore important to more closely investigate the risks of diagnostic radiation-induced injury near implants.

In a previous work we analysed the biological effects of backscatter radiation at tissue–titanium interfaces using tumour therapeutic proton beams and ^{60}Co photons. The colony forming capacity of cultured cells growing as monolayers directly on titanium supports was measured as a function of dose and radiation quality. The cells acted as biological microdosimeters. There were no significant effects when comparing with controls grown on plastic and no overdosage was observed near the titanium surface (10). Biological effects of backscatter radiation from metal surfaces in tissue interfaces using diagnostic quality radiation have, however, not been previously analysed. Dose measurements and calculations (2, 5, 9) indicate that marked biological effects are expected when low energy photons are applied. The aim of the present study was to analyse the biological effects, using the method previously described (10), with the application of low energy photon radiation as is normally used in odontological radiography.

Material and Methods

Cells. Embryonic hamster V79-379A cells (11) were used due to their ability to form well-shaped colonies and their suitability for the clonogenic survival assay. They were chosen only as examples of mammalian cells and not because of their origin.

Culture conditions. Ham's F-10 medium supplement with 10% fetal calf serum, L-glutamine (2 mM), streptomycin (20 $\mu\text{g}/\text{ml}$) and penicillin (20 IU/ml) from Kebo Laboratories AB, Stockholm, Sweden was used. The cells were grown in a standard culture incubator (ASSAB) at 37°C in humidified air containing 5% CO_2 .

Preparation of discs. Titanium discs (commercially pure titanium, grade 1, from Nobelpharma Aktiebolag, Gothenburg), 15 mm in diameter and 5 mm thick, were polished and covered with a very thin layer of palladium (Pd), using a standard evaporation procedure previously described (12, 13). The surfaces of the plastic control discs (Thermanox tissue culture cover slips of 15 mm diameter, LUX 5414, Flow Lab., Stockholm) were also covered with a thin layer of Pd. When applying the standard evaporation method, the thickness of the Pd layer has been estimated to be about 10–50 $\mu\text{g}/\text{m}^2$ (12) and should not significantly disturb the distribution of scattered electrons from the titanium or plastic supports. The Pd layer was used to ensure identical growth conditions for the test cells, independent of the surface conditions of the solid support. Previous studies have shown palladium to be a good support for cultured cells (12–15) and that the Pd layer did not affect the plating efficiency or the radiosensitivity (10).

Seeding on discs. The cells were cultured in conventional 25 cm² flasks (Bibby, Kebo Lab, Stockholm) and repeatedly subcultured through trypsination (0.1% EDTA-trypsin). In preparation for the experiments, cells were subcultured for three passages in flasks at a density allowing for exponential growth. Concentrations of $3.0\text{--}6.0 \times 10^4$ cells were then seeded on either titanium or plastic discs. The discs were placed in conventional 35 mm diameter plastic culture dishes (Bibby, Kebo Lab, Stockholm) with complete medium containing 10% fetal calf serum and antibiotics and incubated under standard culture conditions. The cells were first incubated with only a drop of medium for about 2 h to ensure attachment of the cells to the discs. Thereafter, the culture dishes were filled with the normal amount of medium (2 ml per dish).

Disc holders. Specially manufactured teflon disc holders with teflon-distances and stoppers were, together with plastic culture chambers, sterilized and washed with complete medium. The holders and the culture chambers have previously been described in detail (10). Titanium or plastic discs with V79-379A cells were placed in the teflon holder at a distance of 8 mm from the front surface of the holder. The holder was then placed in the chamber, against the thin wall, with the cell-surfaces towards the x-ray beam. Two holders were placed side by side in the chamber; one containing cells growing in titanium and the other serving as a control and containing cells growing on plastic. The chamber was finally filled with complete medium.

Irradiation. A Siemens Stabilopan x-ray therapy unit with 2 mm Al inherent filtration was used for the experiments. 65 kVp x-rays, with a measured HVL of 1.7 mm Al, were selected to simulate the quality of diagnostic x-ray tubes for intraoral radiographic purposes. The irradiations were made at a distance of 40 cm from the focus. The measured dose rate to water, at the position of the cell-containing discs, was 0.23 ± 0.03 Gy/min. The dose rate was measured with thermoluminescent LiF detectors, which were calibrated in a ⁶⁰Co beam. A factor of 0.7 was applied to the ⁶⁰Co calibration factor, to obtain the dose to water for 65 kVp x-rays. The data from Rudén (16) were used to estimate the value of this factor. The tube current and the irradiation time were used to estimate the dose given to cells in each irradiation. The culture chamber was irradiated under standard incubation conditions (37°C, 5% CO₂) to doses in the interval of 0.7 to 5.0 Gy.

Cloning. Thirty minutes after irradiation the cells were trypsinized, counted in a cell counter (Coulter counter, Industrial model D) and plated for analysis of clonogenic survival (200–100 000 cells/dish depending on the dose) in conventional 8.5 cm culture dishes (Bibby, Kebo Lab, Stockholm). Complete medium was added and the cultures were thereafter incubated at 37°C. The cells (doubling time: $T_d = 14 \pm 2$ h) were allowed to grow as colonies for 6 days before fixation in formalin and staining with haematoxylin. Colonies with more than 50 cells were counted

using an image analyzer (Analytical Measuring Systems, Analytical Instruments Ltd, Sherill, Saffron Walden, Essex, England). The cloning efficiencies of the controls were in the interval of 0.23–0.24.

Analysis of cell survival data. Computer programs, similar to those previously described by Albright (17), were used in the analysis of the cell survival data. The programs fitted the linear quadratic and single-hit multi-target models to the data by iterative, weighted, least square minimization, and the parameters D_0 , n , α and β were calculated. The survival level at the dose 2 Gy ($S_{2\text{Gy}}$) is known to relate to the clinical radiation responsiveness of tumour cells and was calculated from the adapted models. True parameter values could not be obtained in the case of cells on titanium since the dose near the titanium surface was unknown.

Results

The data in the Figure show clear differences in survival of the analysed V79-379A cells after irradiation with 65 kVp x-rays when growing on titanium compared to

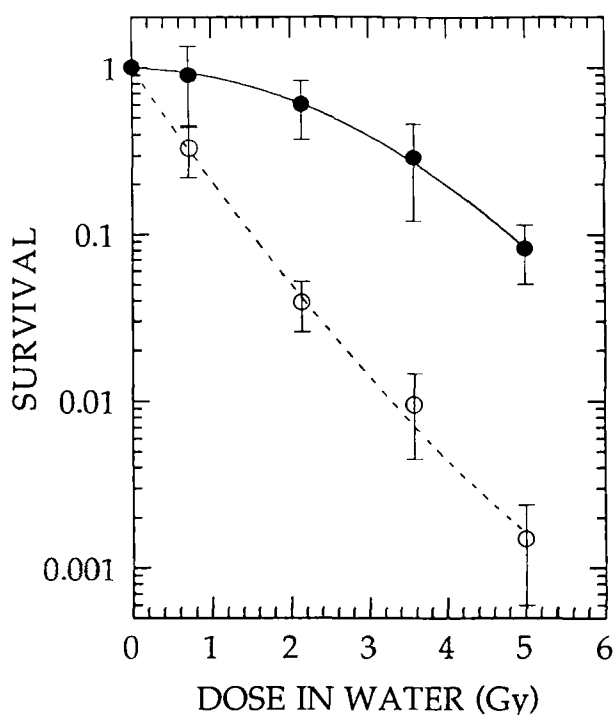


Figure. Cell survival data for the V79-379A cells after irradiation with 65 kVp x-rays. Filled circles are data for cells growing on plastic and open circles for cells growing on titanium during the irradiations. The dose values given on the x-axis are the undisturbed doses given to the cultures when analysed in the culture medium which, from the dosimetric point of view, is equivalent to water. The possibly increased dose near the titanium surface has not been considered since this dose is unknown. Mean values and standard deviations based on 6 independent experiments are given. The solid and dashed lines were obtained from the least square adaption to the linear-quadratic model.

Table

The α , β , D_0 , n and S_{2Gy} values obtained from least square adaption of the experimental data to the linear-quadratic and single-hit multi-target models. GOF is the 'goodness of fit' and low values mean good adaption of the models to the experimental data. The V79-379A cells were grown on plastic or titanium during the irradiation with 65 kVp x-rays. Note that the parameter values are calculated on the basis of the dose in the surrounding culture medium (dose in water) and not on the actual dose near the titanium surface

Support	Linear-quadratic				Single-hit multi-target			
	α (Gy) ⁻¹	β (Gy) ⁻²	GOF	S_{2Gy}	D_0 (Gy)	n	GOF	S_{2Gy}
Plastic	0.0407	0.0905	0.00945	0.62	1.25	4.82	0.0499	0.66
Titanium	1.64	-0.0725	0.137	0.051	0.829	0.584	0.330	0.053

plastic. The survival curve after irradiation on plastic was very similar to the curve previously reported for these cells after irradiation with ⁶⁰Co photons (10). The survival curve after irradiation of cells growing on titanium was much steeper and had no shoulder in the low-dose region. The curve actually resembles a survival curve after irradiation with high LET radiation, such as neutrons or heavy charged particles like carbon or neon ions. Note that the dose on the x-axis is the undisturbed dose in the culture medium surrounding the cells and that the actual dose near the titanium implant is not known.

The experimental data were analysed both with the single hit-multi target and the linear-quadratic models by applying the dose in the surrounding culture medium as the independent variable. The survival curve parameters are shown in the Table. The lines in the Figure are the curves obtained with the linear-quadratic model, which gave the best fits for cells grown both on titanium and plastic. Note that the parameters for the titanium case are not true values and are not comparable with the parameters for the plastic case since the actual dose near the titanium surface is not known.

Discussion

It is, from the presented results, not possible to analyse to what degree the changed shape of the 'titanium curve' in the Figure depended on an increased dose near the titanium implant or on a changed radiobiological effectiveness, RBE. Both factors possibly contribute. An increased dose is expected due to backscattered secondary electrons from the metal surface and the change in shape of the 'titanium curve' indicates that the relative biological effectiveness, RBE, is higher than unity since there is no shoulder in the curve which is a typical feature when RBE increases.

The shape of the survival curve for the V79-379A cells irradiated on plastic with 65 kVp x-rays was similar to the curve obtained previously when ⁶⁰Co photons were applied (10) and it was also similar to the curves that have been previously obtained for V79 cells after irradiation with low LET radiation at other laboratories (18–20). The shape of

the curve obtained after irradiation of the cells when growing on titanium was similar to the type of survival curves often seen after treatment with densely ionizing, high LET radiation, such as neutrons or heavy charged particles (21, 22). It is possible that the secondary electrons which are released from the titanium surface give such a high ionization density that this effect, to some degree, can be compared to the effects of high LET radiation.

The shapes of the curves in the Figure indicate that the obtained relative changes were higher in the low-dose region. The reason is that the survival curve for the cells grown on plastic had a shoulder in the low-dose region while this was not the case for the cells grown on titanium. A similar phenomenon giving higher relative biological effectiveness at lower doses is generally observed after irradiation with high LET radiation, such as neutrons or heavy charged particles.

The changes at low doses seemed to fall within the order of the physical enhancement factors measured or calculated for low energy photons at metal surfaces by other investigators (2, 4, 5, 9). Although previous studies with physical dosimeters have applied different geometries and also other metals, a rough estimate of the expected dose enhancement on a flat titanium surface can be made. For example, the results from Alm-Carlsson (2) concerning the dose enhancement from a metal-tissue equivalent interface are applicable also for the backscatter situation. An interpolation in Figure 11 in the work by Alm-Carlsson, at a distance of 5 μ m from the metal surface (which is relevant for cells grown as monolayers), gives a dose enhancement factor of about 8. Thus, it is possible that a large part of the difference between the two curves in the Figure is due to an increased dose near the titanium surface.

Dosimetric studies for high energy photon beams (23–26) have shown that in this case the dose increase near the tissue titanium surface is 10–15% or higher. In a previous investigation from our laboratory, applying high energy photons or protons (10), we have found that this dose increase could not be seen in the cell survival.

More experiments are necessary with both physical and biological dosimeters on the surfaces of titanium and

applying also other implant metals, such as stainless steel and molybdenum to obtain more detailed knowledge about backscatter radiation. Different radiation qualities should be applied to give knowledge about the importance of both beam energy and the type of the applied radiation for the induction of biologically significant amounts of backscatter radiation. It is possible that variations in the dose rate could help to resolve the question of to what degree the studied phenomena depend on an increased dose near the titanium surface or on a changed relative biological effectiveness.

The doses delivered to patients in diagnostic investigations vary from about 10 μ Gy for single x-ray investigations of teeth up to nearly 1 mGy in more extensive odontological and other intraoral examinations. A diagnostic dose of 1 mGy will, if the results in this study are applied, give biological effects near the titanium implant corresponding to the biological effects in soft tissue of a dose of less than 20 mGy. This is not enough to give acute changes. It is well known that doses of 1 Gy or more are necessary to induce severe acute effects. This can be seen, when clonogenic survival is considered, in the cell survival curve for cells on plastic in the Figure in this article and it can also be deduced from basic radiobiological data in the literature. Furthermore, an enhancement factor of 20 is lower than the estimations previously made by Stenström (5). Thus, the biological measurements described here give a lower enhancement factor than previously estimated and this supports the use of intraoral radiography postoperatively after titanium implantations without the risk of getting additional healing disturbances.

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