# Tissue response of radiation therapy assessed by electrical impedance spectroscopy (EIS) in subcutaneous tumours in rats.

Persson, B. R. R.; Bauréus Koch, C.; Grafström, G.; Salford, L. G.

*Published in:*
Acta Scientiarum Lundensia

2021

*Document Version:*
Publisher's PDF, also known as Version of record


**Volym ASL 2021-002**

**Citation: (Acta Scientiarum Lundensia)**

**Persson B.R.R. et. al (2021).** Tissue response of radiation therapy assessed by electrical impedance spectroscopy (EIS) in subcutaneous tumours in rats. ***Acta Scientiarum Lundensia*** **ISSN1651-5013, Vol. 2021-002 pp. 1-19**

## Corresponding author:

Bertil R.R. Persson PhD, MDhc
Professor emeritus of medical radiation physics
Medical Radiation Physics Department
Lund University Hospital
Barngatan 4
SE 22185 LUND, Sweden

E mail: Bertil_R.Persson@med.lu.se

## Lund 2021

Acta Scientiarum Lundensia ASL 2021-002 2021-04-30
(open acess)

# Tissue response of radiation therapy assessed by electrical impedance spectroscopy (EIS) in subcutaneous tumours in rats

B. R. R. Persson[1,3], C. Bauréus Koch[1,3], G. Grafström[1,3], and L. G. Salford[2,3],
[1]Dept. Medical Radiation Physics, [2]Dept. Neurosurgery,
[3]The Rausing Laboratory, Lund University, S-221 85 Lund, Sweden

**Abstract**

The present investigation aims to evaluate the possibility of using bio-impedance spectrometry to measure tumour and tissue response to radiation therapy. Bio-impedance measurements performed with CythorLab™ equipped with a signal generator with a known high output impedance and signal measuring device able to measure the voltage applied by the generator. The control unit triggers the signal generator that generates an MLS-sequence. The same control unit process the signal that simultaneously measured by the voltage-recording device. An FFT analysis performed to obtain the magnitude of the real and imaginary parts of the impedance spectrum.

The effect of various numbers of fractions of radiation therapy (RT) on the impedance measured with surface plate electrodes investigated in male rats of the Fischer-344 strain with rat glioma N32 tumours implanted subcutaneously on the flank. Tumours produced by injecting 100 000 N32 tumour cells just below the skin. Tumours were treated about four weeks after injection when a solid tumour has developed with a diameter of 1-1.5 cm. Before treating the tumours, animals anaesthetised, and the fur over the tumour shaven and carefully to ensure good electrical contact between electrodes and skin.

The electrical impedance dispersion of tissue modelled with an RC-equivalent circuit from which collective impedance parameters corresponding the cell membranes, $R_m$ $C_m$ intra-cellular resistance, $R_i$, and extra-cellular resistance R0.

Impedance measurements performed over a tumour before irradiation to 5 Gy and every minute after the irradiation up to 8 minutes. A slight increase of impedance, and with a time constant of 10 minutes. The growth might be due to dry skin after irradiation or a decrease of tumour vascularity during the treatment.

The capacitance of the cell membrane related to the characteristic frequency fc does not change significantly before and after radiation exposure. A special parameter, the "Loss Change Index" (LCI) which defined to vary between zero if there is no change in the phase angle and one if the phase angle after exposure approach zero. LCI reach an extreme at the characteristic frequency. The LCI value recorded at the characteristic frequency fc varied with the accumulated absorbed dose and fitted to a sigmoidal dose/response relationship.

***Keywords:*** radiation therapy, bio-impedance spectrometry, tissue response, CythorLab™, rat glioma, N32 tumours, subcutaneous, 60Co-gamma irradiation, RC-circuit model, cell membranes, dose/response relation.

Bertil_R.Persson@med.lu.se

**Contents**



Bertil_R.Persson@med.lu.se

# 1. Introduction

## 1.1. *Therapeutic response of radiation therapy*

Radiation therapy is still the most widely used therapy modality for cancer treatment, although there is a lot of progress made in developing new methods and techniques for cancer. It is a complex task to evaluate the therapeutic effects of new treatment modalities and their combinations and compare them with conventional radiation treatment. In a previous study, we were the first to show that treatment of N32 tumours with pulsed electric fields sensitise the tumour to radiation damage ( Engström et al 2001). A model developed for evaluating the therapeutic response of combined treatment modalities applied to *in vivo* studies of cancer treatment by using pulsed electric fields combined with 60Co-gamma radiation therapy (Persson et al. 2003, 2004).

The present investigation aims to evaluate the possibility of using bio-impedance spectrometry in the frequency range of 1-100 kHz to measure the acute tissue response from radiation therapy. There are some previous reports on bio-impedance spectrometry of tumours after radiation therapy.

## *1.2 Dielectric response of tissue irradiation*

Nuutinen et al. (1998) measured the dielectric constant of irradiated human skin to test the dielectric measurements' feasibility in quantising acute and late radiation reactions. The dielectric constant of irradiated breast skin was measured at an electromagnetic frequency of 300 MHz in 21 patients during post-mastectomy radiotherapy. The measurements performed with an open-ended coaxial line reflection method. The irradiation technique consisted of an anterior photon field to the lymph nodes and a matched electron field to the chest wall using conventional fractionation of five fractions per week to 50 Gy. Fourteen out of the 21 patients were re-measured two years later, and the skin palpated for subcutaneous fibrosis. At five weeks, the dielectric constant had decreased by 31 and 39% for the photon and electron fields' investigated skin sites, respectively.

There was a statistically significant inverse correlation between the mean dielectric constant and the clinical score of erythema. An unexpected finding was a decrease in the dielectric constant of the contralateral healthy skin during radiotherapy. A statistically significant positive correlation was found two years later between the dielectric constant at the irradiated skin sites and the clinical score of subcutaneous fibrosis. Dielectric measurements non-invasively yield quantitative information concerning radiation-induced skin reactions (Nuutinen et al. 1998).

## *1.3 Modelling RC-circuit schemes to in vivo electrical impedance measurements*

Osterman et al. (1999) applied several modelling schemes to *in vivo* electrical impedance measurements on irradiated and normal muscle in rats' hind legs. Specifically, seven-parameter parallel pathways and embedded membrane circuit models have been fit to group averages of impedance spectra measured at different doses and time points. Correlations between histological scored tissue sections and model parameters have also determined. The results show that both models produce good fits to the experimental observations, especially in irradiated tissues. The correlations between histology scores and circuit parameters were, however, higher with the embedded model. Trends in the spectra model parameters agree with the expected changes in tissue pathophysiology

associated with the progression of tissue injury from radiation exposure. Quantitative correlations with specific histological criteria were less conclusive, suggesting a relation to the types and extent of tissue damage induced by radiation treatments (Osterman et al. 1999).

Pigott et al. (2000) describe a system for recording the electrical conductance of skin as a measure of sweat gland function. In 22 normal volunteers close agreement was obtained between measurements obtained from comparable sites on both sides of the chest. Measurements were subsequently made in 38 patients treated by radiotherapy to one side of the chest for tumours of the breast or lung using one of five different fractionation schedules. Simultaneous readings were obtained from both sides of the chest with the non-irradiated side acting as a control.

A dose-response relationship in five patients who received the equivalent total dose of 15 Gy in 2-Gy fractions showed no change in conductance. Sixteen out of 23 who received a total absorbed dose of 42-46 Gy in 2-Gy fractions had a greater than 22% reduction in mean skin conductance than that of the control areas despite the skin appearing normal in the large majority. Marked changes in skin conductance observed after higher total doses. In a prospective study 18 women receiving breast, irradiation underwent weekly readings during treatment. A mean reduction of 40% in skin conductance observed at the end of the second week of treatment before any clinical evidence of radiation change. Skin conductance returned to normal in 44% of patients by 6 months. In the remainder, those patients who showed the most significant reduction in skin conductance during treatment demonstrated a minor recovery. Changes in sweat gland function can be detected and quantified in the skin, which may otherwise appear normal. Differences occurred between areas treated using different fractionation schedules and the method applied to examine the sensitive patient (Pigott et al. 2000).

In the present work, an equivalent RC-circuit model of the tumour tissue will be used to evaluate the response of impedance parameters in subcutaneously implanted tumours in rats after applying pulsed electric fields and irradiation with 20 Gy cobalt-60 gamma radiations in fractions of 5 Gy per day, pulsed electric fields and their combinations.

## 2. Material and methods

### *2.1 Animals*

Inbreed Fischer-344 rats of both sexes weighing around 280 ± 90 g. The strain maintained by continuous, single-line brother/sister mating in our laboratory During the experiment, the animals were housed in a climate-controlled cabinet and stored in *Macralon* cages provided with food pellets and water *ad libitum*. The Animal Ethical Committee approved all experimental animal procedures in Malmö/Lund (Lunds tingsrätt, Box 75, 22100 Lund Sweden).

### *2.2. Tumour cell culture and subcutaneous inoculation.*

The rat glioma N29 induced by trans-placental administration of ethyl-N-nitrosourea to 17- to 18-days pregnant Fischer rats. By this method 80-90% of the offspring developed tumours in the central or peripheral nervous system. N29 arose at 205 days after induction in the right hemisphere of a female offspring.

The tumour is only weakly immunogenic and grows readily intracerebrally, subcutaneously and *in vitro* (Siesjö et al. 1993).

All cells were cultured in antibiotic-free RFMI-1640 medium supplemented with 5-10% fetal calf serum, 2 mM L-glutamine, 10 mM HEPES, 0.5 mM pyruvate and 0.096% NaHCO3. All cell-cultures regularly checked for contaminating microbes by staining with the fluorescent dye Hoechst 32 258 and were examined with fluorescent microscopy. Cultures suspected of Mycoplasma infection were eliminated or treated with Mycoplasma Removal Agent (Hoechst , Germany). Twice with 7 days interval, and repeatedly confirmed free of infection as above. Cell cultures were maintained in culture flasks (Nunc, Denmark) and harvested by treatment with trypsin/EDTA.

The rat glioma N29 was induced in our laboratory by subcutaneous administration in the hind legs. Into the right leg, 200 000 cells inoculated, whilst 50 000 cells inoculated into the left leg, simulating metastasis. Tumour volume measured with a calliper as an ellipsoid by length, width and thickness. When the tumours reached a volume of 9 $cm^3$, the animal sacrificed for ethical reasons.

### *2.3 Radiation treatment (RT)*

The animals anaesthetised i.p.. with 5% chloral hydrate or Ketalar® /Rompun®, 0.55 ml per 100g body weight, Then given fractionated radiation treatment of 5 Gy per fraction, using a 60Co radiotherapy unit (Siemens Gammatron S) with a source-skin distance (SSD) of 80 cm and the maximum absorbed dose rate 0.65-0.70 Gy/min. A 0.5 cm thick, tissue-equivalent bolus (Super Flab, Mike Radio-nuclear instruments inc. NY, USA) was placed over the tumour to achieve full dose build-up A homogeneous dose distribution in the tumour. The radiation field size was collimated to cover the tumour area with a margin of at least 1 cm (Fig 2-1). The absorbed dose was measured using a direct patient dose monitor (DPD6, Instrument AB Therados, Uppsala, Sweden) and a diode probe (Type A for 60Co) placed next to the tumour the field under the bolus.

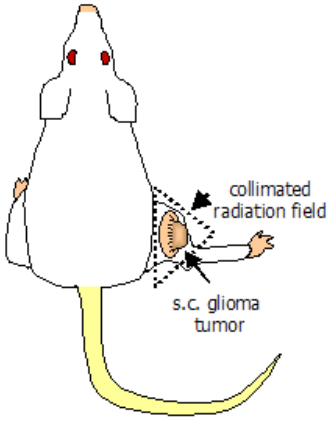


**Figure 2-1** Experimental set-ups for radiation treatment of subcutaneous tumours

## *2.4 Electrical-impedance spectrometry (EIS)*

### 2.4.1 RC-circuit model

Bio-impedance measurements performed with CYTHORLAB™(ADITUS MEDICAL AB, LUND, SWEDEN) that equipped with a signal generator with a known high output impedance and a signal measuring device able to measure the voltage applied by the generator. The control unit triggers the signal generator that generates an MLS-pulse sequence of 0.75 mA and 16 ms long. The same

control unit process the signal that simultaneously measured by the voltage-measuring unit. An FFT analysis performed to obtain the magnitude of the impedance spectrum's real and imaginary parts.

Various parameters in an RC-circuit model of the HeLa cells in suspension evaluated applying electric pulses. Figure 2-2 shows the parallel resistance-capacitance RC-model, where the extracellular space represented by the resistance R*e,* and the intracellular space by the resistance R*i*. The cell membrane represented by capacitance C*m*, and membrane resistance R*m* .

By measuring the impedance spectra in tissue before and after irradiation, the impedance values estimated from the equivalent RC-circuit model shown in Figure 2-2.

Before irradiation, it assumed that collective resistance $R_M$ of the intact membrane is very large compared to the intracellular resistance $R_I$ and resistance of the extracellular space $R_E$ . The impedance values at infinite frequency $Z_¥$ and zero frequency $Z_0$ (DC), respectively, can thus be estimated by the following equations: treatment of subcutaneous tumours.

$$\lim_{\varpi \to 0} Z_{cellsusp} \to Z_0 = \frac{R_E \cdot (R_I + R_M)}{(R_I + R_M + R_E)} \xrightarrow[R_M >> R_I ; R_E]{before} Z_0 \approx .$$

$$\lim_{\varpi \to \infty} Z_{cellsusp} \to Z_\infty = \frac{R_E \cdot R_I}{R_I + R_E}$$

The characteristic frequency given by the equation

$$f_c = \frac{R_E + R_I + R_M}{2\pi \cdot R_M \cdot (R_E + R_I) \cdot C_M}$$

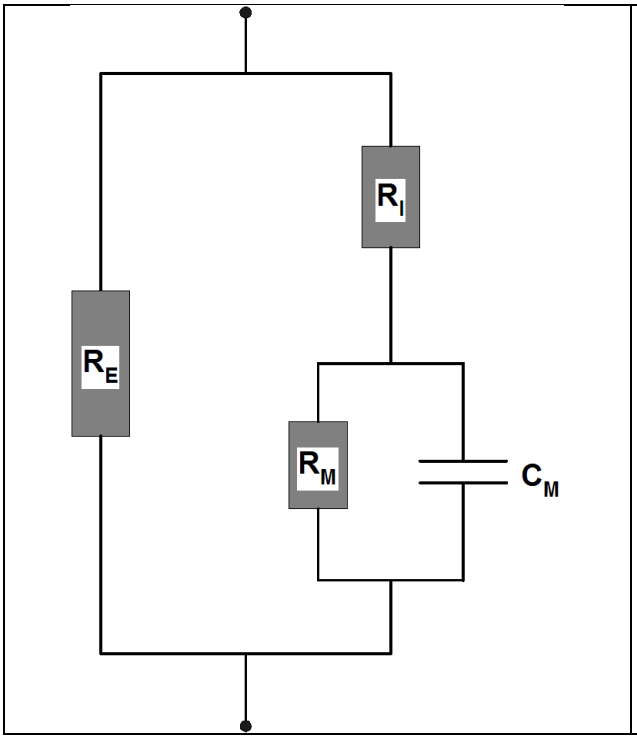


**Figure 2-2.**

Collective equivalent RC-circuit model of tissue. The extra-cellular space represented by the resistance $R_E$, and the collective intracellular space by the resistance $R_I$. The cell membranes represented by the collective resistance $R_m$ and capacitance $C_M$,

The information obtained about the tissue's impedance characteristics measured the impedance spectrum in the frequency domain 1 – 100 kHz. At frequencies below 500 Hz, the measurements are influenced by electrode polarisation processes and higher frequencies, above 100 kHz, by the inductance of the cable connections between the electrodes and the measuring device.

The impedance values at infinite frequency *Z¥* and zero frequency *Z0* (DC), estimated by fitting the *in vivo* bio-impedance data in the frequency interval 1-100 kHz with the following logistic expression of the impedance.

$$Z = Z_{\infty} + \frac{Z_0 - Z_{\infty}}{1 + \left(\frac{f}{f_c}\right)^q}$$

where $Z_c$ the impedance value at $f_c$ the frequency half way between the two limiting values $Z_\infty$ and $Z_0$

$$Z_c = \frac{Z_0 - Z_{\infty}}{2}$$

The frequency $f_c$ at the inflexion point is related to q according to the following expression:

$$\left(\frac{f_1}{f_c}\right)^q = \frac{q-1}{q+1}$$

The tissue modelled as a parallel RC circuit (Figure 2-2), where C*m* is a capacitor with losses. The loss angle δ of a capacitor is defined so that the ideal capacitor with zero losses has zero loss angle (δ = 0). This means that δ = 90° - φ, where φ is the recorded phase angle. The loss tangent, tan δ, is also called the *dissipation factor* equivalent to the energy loss per cycle divided by the energy stored per cycle (r.m.s or peak value). Radiation effects induce losses which increase the loss angle and thus the loss tangent, tan δ. The imaginary part chosen to be negative, and therefore, the loss tangent is increasingly negative.

$$\tan\varphi = \frac{\mathrm{Im}\,Z}{\mathrm{Re}\,Z} \qquad \text{and} \qquad \tan\delta = \frac{\mathrm{Re}\,Z}{\mathrm{Im}\,Z} = \cot\varphi$$

The power loss in the circuit only occurs in the resistive part Re Z if the capacitive element is considered an ideal capacitor. With constant amplitude current, the power loss goes from a constant value at very low frequencies through a maximum at the characteristic frequency fc = 1/RC and to zero at high frequency.

The change of the loss tangent measured at the characteristic frequencies before and after electroporation used as a dosimetry quantity for evaluating the effect of electroporation. This quantity is called the "Loss Change Index" (LCI) and is defined as follow:

$$\textit{Loss Change Index} = \left(1 - \frac{\cot\varphi_{before}}{\cot\varphi_{after}}\right) = \left(1 - \frac{\tan\varphi_{after}}{\tan\varphi_{before}}\right) = \left(1 - \frac{\mathrm{Im}\,Z_{after} \cdot \mathrm{Re}\,Z_{before}}{\mathrm{Re}\,Z_{after} \cdot \mathrm{Im}\,Z_{before}}\right)$$

### 2.4.2 Model-free evaluation

The recorded impedance spectra's real and imaginary parts vary differently in various frequency intervals after irradiation subcutaneous tumours. The average change in the absolute value ΔZ%, real part $\Delta\Re\%$ , imaginary part $\Delta\Im\%$ argument Δθ%, (ARG) and Δtanθ% studied in the frequency intervals 1kHz - 10 kHz and 10kHz and 100 kHz.

$$\Delta\Re\% = \frac{100}{n}\cdot\sum_{1kHz}^{10kHz}\frac{\mathrm{Re}\,Z_{before}-\mathrm{Re}\,Z_{after}}{\mathrm{Re}\,Z_{before}};\qquad \Delta\Im\% = \frac{100}{n}\cdot\sum_{10kHz}^{100kHz}\frac{\mathrm{Im}\,Z_{before}-\mathrm{Im}\,Z_{after}}{\mathrm{Im}\,Z_{before}}$$

$$\Delta\theta^{\Re} = \frac{100}{n}\cdot\sum_{1kHz}^{10kHz}\frac{\theta^{\Re}_{before}-\theta^{\Re}_{after}}{\theta^{\Re}_{before}};\qquad \Delta\theta^{\Im} = \frac{100}{n}\cdot\sum_{10kHz}^{100kHz}\frac{\theta^{\Im}_{before}-\theta^{\Im}_{after}}{\theta^{\Im}_{before}}$$

$$\Delta\tan\theta^{\Re} = \frac{100}{n}\cdot\sum_{1kHz}^{10kHz}\frac{\tan\theta^{\Re}_{before}-\tan\theta^{\Re}_{after}}{\tan\theta^{\Re}_{before}};\qquad \Delta\tan\theta^{\Im} = \frac{100}{n}\cdot\sum_{10kHz}^{100kHz}\frac{\tan\theta^{\Im}_{before}-\tan\theta^{\Im}_{after}}{\tan\theta^{\Im}_{before}}$$

Where n is the number of data in the corresponding intervals

All these parameters thus tested model-free quantities for studying the response of RT in superficial tumors.

*2.5. Curve fitting and Statistics*

The dose response data fitted to a logarithmic dose response equation with variable hill slope, *k* (ORIGIN PRO 7, OriginLab.com, USA).

$$y = A_0 + \frac{A_\infty - A_0}{1+10^{(x_c-x)\square k}}$$

where

$A_0$ is the bottom asymptote
$A_\infty$ is the top asymptote
$x_c$ is the center x value i.e. at $y = (A_\infty - A_0)/2$
*k* is the hill slope,

The correlation coefficient (r) represents the linear relationship between two variables. If the correlation coefficient is squared, then the resulting value ($r^2$, the *coefficient of determination*) will represent the proportion of common variation in the two variables (i.e., the "strength" or "magnitude" of the relationship). In evaluating the correlation between variables, it is essential to know this "magnitude" or "strength" and the significance of the correlation. The *coefficient of determination,* $r^2$, gives a measure for the goodness of fit of parameters to exponential and dose-response functions.

Bertil_R.Persson@med.lu.se

## 3. Results of Radiation Therapy

The effect of various numbers of radiation therapy fractions on the impedance measured with surface plate electrodes investigated in male rats of the Fischer-344 strain with rat glioma N32 tumours implanted subcutaneously on the flank. Tumours produced by injecting 100 000 N32 tumour cells just below the skin. Tumours were treated about four weeks after inoculation when a solid tumour has developed with a diameter of 1-1.5 cm. Before pulse treatment of the tumours, animals were anaesthetised. The fur over the tumour was shaven and carefully covered with electroconductive paste to ensure good electrical contact between electrodes and skin.

### *3.1 Tumour Growth Rate*

Animals arranged into five groups. One group of 10 controls (C) received no treatment. In the other four groups, $^{60}$Co-gamma irradiation (5 Gy per session) given with one, two, three and four daily treatments. The number of rats appears above each respective column in **Figure 3-1**.

The length, width and thickness of the tumour was measured daily during the week and estimating the tumour volume as an ellipsoid evaluated tumour volume. The tumour volume data of each animal fitted to an exponential tumour growth model. Thus, the mean time t1 of tumour growth and tumour growth rate, the inverse of t1 (TGR=1/ t1), was evaluated for each rat. The average TGR of each group of animals calculated and displayed in **Figure 3-1**, showing the tumour growth rate (TGR % per day) of Controls and tumours treated 1, 2, 3 and 4 sessions with radiation therapy 5 Gy per day. The number of animals in each treatment group appears in the respective column. The value of significance from the t-test of growth rate versus controls at each column shows where significance appears. The lowest growth rate appears after one treatment of 5 Gy.

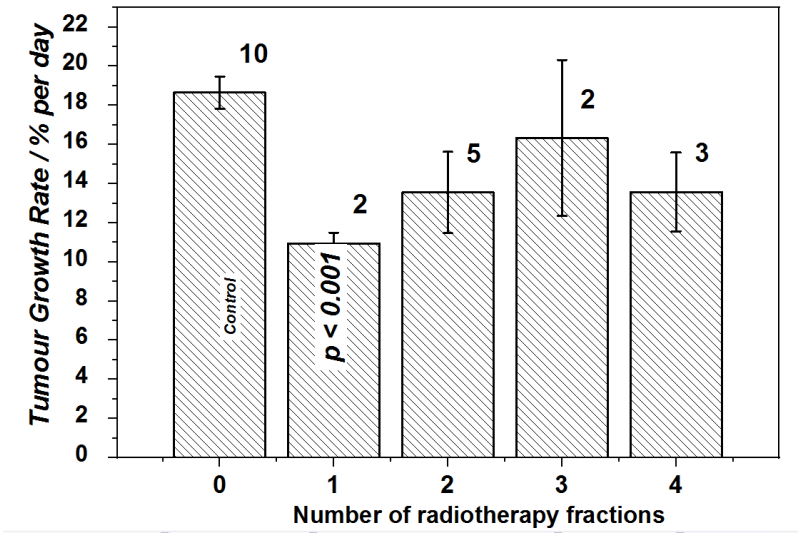


**Figure 3-1**

The histogram shows the TGR of controls and tumours treated 1, 2, 3 and 4 sessions with radiation therapy 5 Gy per day (totally 5, 10, 15, and 20 Gy ). The numbers of animals in each treatment group are given at each column respectively.

Bertil_R.Persson@med.lu.se

*3.2 Impedance measurements*

The evaluation of impedance model parameters before and 1 min after exposure to various RT fractions of $^{60}$Co gamma radiation of 5 Gy appears in **Table 3-1.**

**Table 3-1**
Results of evaluation of impedance model parameter ratios before and 1 min after exposure to 1 - 4 fractions $^{60}$Co gamma radiation of 5 Gy. Each value is the average of 4 animals ± SE.

| Treatment No. | $Z_o$ | p-value | $R_M$ | p-value | $Z_0/R_M$ |
|---|---|---|---|---|---|
| 0 | 1 | | 1 | | ? |
| 1 | 0.59 ± 0.09 | 0.01 | 0.021 ± 0.007 | <0.00001 | 96 ± 39 |
| 2 | 0.39 ± 0.11 | <0.001 | 0.006 ± 0.002 | <0.00001 | 549 ± 264 |
| 3 | 0.23 ± 0.06 | <0.0001 | 0.003 ± 0.001 | <0.00001 | 1763 ± 1144 |
| 4 | 0.13 ± 0.08 | 0.01 | 0.002± 0.001 | <0.00001 | 3018 ± 2245 |

| Treatment No.. | $Z_\infty$ | p-value | $R_I$ | p-value | $C_M$ | p value |
|---|---|---|---|---|---|---|
| 0 | 1 | | 1 | | 1 | |
| 1 | 1.66 ± 0.27 | N.S. | 1.70 ± 0.27 | N.S. | 1.20 ± 0.53 | N.S. |
| 2 | 0.60± 0.15 | 0.03 | 0.83 ± 0.34 | N.S. | 1.10 ± 0.31 | N.S. |
| 3 | 0.96± 0.35 | N.S. | 1.00 ± 0.37 | N.S. | 1.52 ± 0.55 | N.S. |
| 4 | 0.99 ± 0.34 | N.S. | 1.07 ± 0.40 | N.S. | 1.16 ± 0.49 | N.S. |

After the first fraction, the most significant decrease was in the resistance RM of the membrane. The impedance values value at infinite frequency Z¥ and the intracellular resistance RI increased, while the impedance values value at zero frequency Zo decreased. The cell membrane's capacitance that derived from the following relation to the characteristic frequency does not change significantly before and after radiation exposure.

$$C_M = 1/[2\pi \cdot (Z_0 + R_I) \cdot f_c]$$

where $f_c$ is the characteristic frequency at $(Z_0 + Z_\infty)/2$.

As can be seen from **Table 3-1,** after the 2nd fraction, there is a significant decrease in the impedance at both zero $Z_0$ and infinite frequency, $Z_\infty$ as well in the collective membrane resistance $R_M$, but no substantial change in the intracellular resistance $R_I$ or capacitance of the cell membrane $C_M$. After the 3$^{rd}$ and 4$^{th}$ fractions, there is a significant decrease in the DC impedance at zero frequency $Z_0$ and in the membrane resistance $R_M$.

The change in the membrane resistance $R_M$, before and after RT decreases exponentially between each fraction of radiation. This change might due to the oxidation of the lipids by the free

radicals of the irradiation, forming reactive oxidised species (ROS) that destabilise the membranes and makes them leakier.

The ratio $R_0/R_M$ named "impedance index", appears as a parameter independent of the electrodes and measuring condition, This parameter is displayed in **Figure 3-2** together with the values of $R_0$ and $R_M$, respectively, that exhibit an interesting "pharmacological" dose/response relationship.

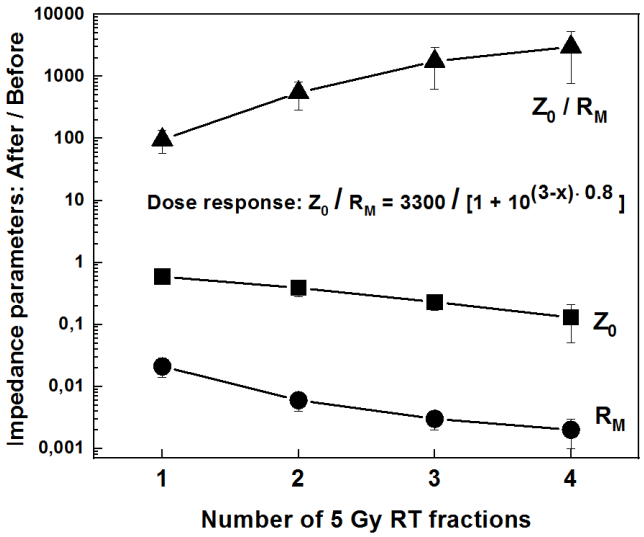


**Figure 3- 2**
The values of the ratios of the DC impedance at zero frequency $Z_0$ and in the collective membrane resistance $R_M$ and their ration $Z_0/R_M$ after various number of 5 Gy RT fractions.

There is an exponential increase in the DC impedance **Zo** with time after irradiation that might occur due to the cells' swelling. The data fit very well ($r^2$ =0.98) to a dose-response equation starting at 95000 Ω with a final value of 207 830 Ω and raised to 50% at about 5 min after irradiation

$$\mathbf{Z_o(t)} = 9500 + 112830 / (1 + 10^{(\mathbf{5.1-t})\cdot\mathbf{0.28}}),$$

## *3.3 Loss Change*

The loss tangent ratio at a specific frequency after and before tissue exposure indicates a change of dielectric properties and measure its transformation in the lossy compartment. This ratio can thus be used as a dosimetry parameter to relate to the effect of radiation exposure. The "Loss Ratio" is evaluated as follow:

$$Loss\ \text{Ratio} = \left( \frac{cot\theta_{after}}{cot\theta_{before}} \right)$$

The Loss Change is equal to 1 if there is no change in the phase angle, and the maximum value applies to measure the effect of the exposure.

. In extreme powerful exposure, the exposed object's loss factor tends to be infinitely high, and the Loss Ratio is not defined. Therefore another quantity is determined, the "Loss Change Index" (LCI) described as follow:

$$Loss\ Change\ Index = \left(1 - \frac{cot\theta_{before}}{cot\theta_{after}}\right)$$

By this definition, the Loss Change Index varies between zero if there is no change in the phase angle and equals 1 when the phase angle approaches 0 and the Loss tangent approach infinity.

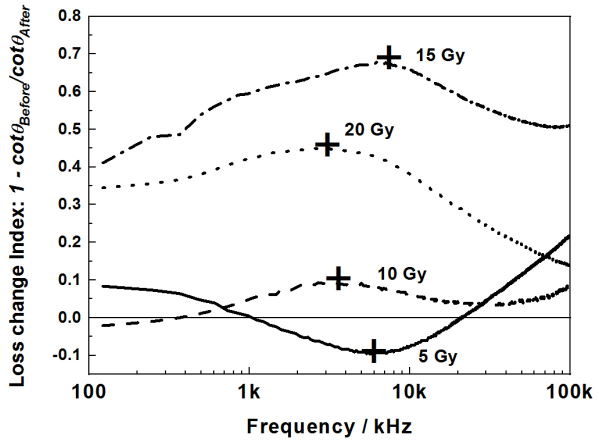


**Figure 3-3**

The "Loss Change Index" (LCI) calculated over the frequency interval of 0.1 - 100 kHz for subcutaneous tumours treated with 5 Gy fractions of radiation RT at 1-4 days.

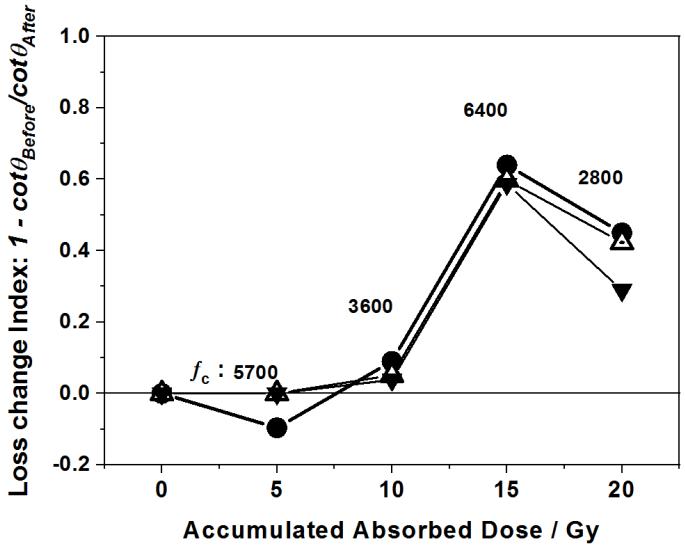


**Figure 3-4**

Loss Change Index at various accumulated absorbed dose. Open circles ○) represents the extreme values at frequencies given in the figure, ▼ represent the values at 110 Hz, and △ represents the values at 20 700 Hz (rat 5181 d1-d4 / rbrp 020728)

### *3.4 Model-free analysis*

T Recorded impedance spectra's real and imaginary parts vary differently in various frequency intervals after irradiation subcutaneous tumours. The change of the real part, $\Delta\Re\%$, varies, however, smoothly between 1kHz and 10 kHz and the average change of the imaginary part, $\Delta\Im\%$, between 10 kHz and 100 kHz. The average change in the absolute value $\Delta Z\%$, real part $\Delta\Re\%$, imaginary part $\Delta\Im\%$ argument $\Delta\theta\%$, (ARG) and $\Delta\tan\theta\%$ was studied in the frequency intervals 1kHz - 10 kHz and 1okHz and 100 kHz. All these parameters are thus tested model-free quantities for studying the response of radiation RT in superficial tumours. The best correlation found in for the imaginary part $\Delta\Im\%$ in the frequency interval 1kHz - 10 kHz, and argument $\Delta\theta\%$, and $\Delta\tan\theta\%$ in the frequency interval 10kHz - 100 kHz. These data are displayed in figure 3-5 and fitted to the logarithmic dose-response function. Top asymptote values, x value for centre level and the slope k given in table 3-2. The 50% level close to 2, and the slope is close to 1.0 for all three parameters. But the highest asymptotic value of 60% is found for the imaginary part in frequency interval 1-10 kHz.

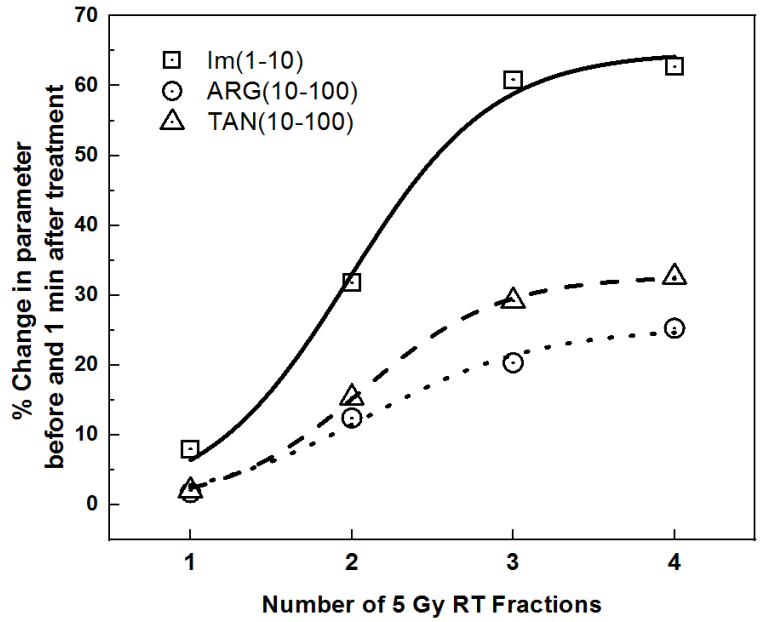


**Figure 3-5**

The imaginary part $\Delta\Im\%$ in the frequency interval 1kHz - 10 kHz, argument $\Delta\theta\%$, and $\Delta\tan\theta\%$ in the frequency interval 10kHz - 100 kHz. These data fitted to the logaritmic dose-response function:

$$Y = A_\infty / (1+ 10^{(x_c - x)\cdot k}) .$$

Table 3-2 The parameters of the data fitted to the dose response function $Y = A_\infty / (1+ 10^{(x_c - x)\cdot k})$

| Parameter | $A_\infty$ Top asymptote | $X_c$ | k | r2 |
|---|---|---|---|---|
| $\Delta\Im\%$ 1 - 10 kHz | 64.8 ± 3.3 | 1.98 ± 0.10 | 0.98 ± 0.21 | 0.995 |
| $\Delta\theta\%$ 10-100 kHz | 25.3 ± 2.5 | 2.10 ± 0.20 | 0.82 ± 0.27 | 0.989 |
| $\Delta\tan\theta\%$ 10-100 kHz | 32.7 ± 0.7 | 2.06 ± 0.04 | 1.04 ± 0.09 | 0.999 |

Bertil_R.Persson@med.lu.se

## *3. 5 Kinetic change of impedance parameters after irradiation*

A tumour implanted at the flank of Rat #5161 was irradiated with 5 Gy Co-60 gamma radiation. Impedance measurements performed before and stopped up every minute after the irradiation to 8 minutes.

**Figure 3-6** shows the impedance at infinite frequency $Z_\infty$ obtained by fitting the impedance spectra recorded over the tumour to a sigmoid function. There seems to be a slight increase of $Z_\infty$ over time but with a large spread in the values.

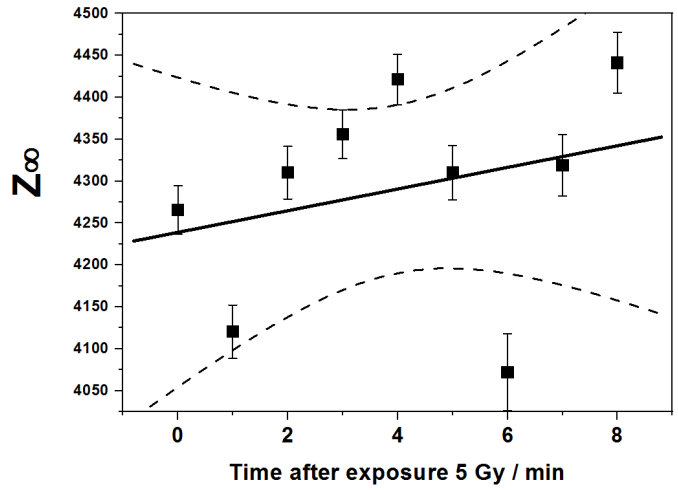


**Figure 3-6**

Time variation of the infinite impedance $\mathbf{R_\infty}$ measured over the tumour after exposure with 5 Gy.

*5161 2000-09-18 $R_\infty$*

**Figure 3-7** shows the DC impedance $\mathbf{Z_0}$ obtained by fitting the impedance spectra recorded over the tumour to a sigmoid function. There is an exponential increase in the DC impedance $\mathbf{Z_0}$ over time with a time constant of 10 minutes.

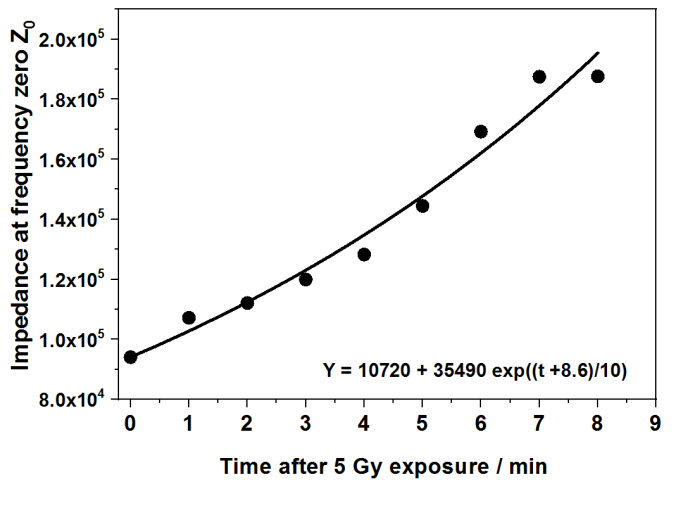


**Figure 3-7**

Time variation of the DC impedance $\mathbf{Z_0}$ measured over the tumour after exposure with 5 Gy.

# 4. Discussions and conclusion

## *4.1 Variation of impedance model parameters with absorbed dose*

The effect of various numbers of radiation therapy fractions on the impedance, measured with surface plate electrodes, was investigated in male rats of the Fischer-344 strain with rat glioma N32 tumour implanted subcutaneously on the flank. The tumour growth rate (TGR % per day) of controls and tumours treated 1, 2, 3 and 4 sessions respectively with radiation therapy 5 Gy per day indicate that one single treatment of 5 Gy with $^{60}$Co gamma radiation caused a significant decrease ($p<0.01$) in the growth of the tumour. The second and the 3$^{rd}$ session cause a growth rate increase, but after the 4$^{th}$ session, the growth rate decrease again.

On day one, before and 1 min after exposure to the 1$^{st}$ fraction $^{60}$Co gamma radiation of 5 Gy, the most significant changes occur in the resistance $R_M$ of the membrane and the impedance at zero frequency. The collective capacitance of the cell membranes does not change significantly before and after radiation exposure.

On day two, before and 1 min after exposure to the 2nd fraction $^{60}$Co gamma radiation of 5 Gy, the same pattern occurs as after the first fraction. A significant decrease in the impedance occurs at infinite frequency.

On day three, before and 1 min after exposure to the second fraction $^{60}$Co gamma radiation of 5 Gy, the same pattern occurs after the first fraction.

On day four, before and 1 min after exposure to the 4$^{th}$ fraction (totally administered dose 20 Gy, the same pattern occurs as after the first fraction.

The ratio for the various model parameters 1 min after and before the radiotherapy session shows that the impedance parameters recover after each fraction of radiation. Exceptions are the DC impedance at zero frequency and the membrane resistance $R_m$, which decrease exponentially between each fraction of radiation. This change might due to the oxidation of the lipids by the free radicals of the irradiation, forming reactive oxidised species (ROS) that makes the membranes leakier.

The ratio $R_0/R_m$ (Impedance index) is exhibit the following interesting "pharmacological" does/response relation

$$(y = 0.01 + 3301/ (1 + 10^{(14.4-x)*0.162}).$$

The Impedance index might be a suitable quantity for assessing the effects of radiation in tissue by impedance measurements..

## *4.2 Kinetic change of impedance parameters after irradiation*

Impedance measurements over a tumour were performed before irradiation to 5 Gy and every minute after the irradiation were stopped up to 8 minutes. There seems to be a slight increase of $Z_\infty$ over time but with a large spread in the values and an exponential increase in the DC impedance $\mathbf{Z_0}$ over time with a time constant of 10 minutes. The increase might due to that the skin gets dry after irradiation or to decrease of tumour vascularity during the treatment.

The decrease of the relative difference between $\mathbf{Z_0}$ and $\mathbf{Z_\infty}$ the so called $\mathbf{Py = ( Z_0 - Z_\infty)/ Z_0}$ is often used as an index of tissue damage. After radiation however the $P_y$ index increases probably because of

the same reason as the increase in $\mathbf{Z_0}$.

## *4.3. Change in the loss tangent or dissipation factor*

The ratio between the loss tangent before and after exposure of tissue is a measure of the tissue's radiation response. This ratio can be used as a dosimetry parameter to relate to exposure to ionising radiation.

The "Loss Ratio" is evaluated as follow: $Loss\ \text{Ratio} = \left( \frac{cot\theta_{after}}{cot\theta_{before}} \right)$

The Loss Ratio is 1 if there is no change in the phase angle and varies with frequency, and the maximum value applies as a measure for the effect of the exposure.

In extreme powerful exposure, the exposed object's loss factor tend to be infinitely high, and the Loss Ratio is not defined. Therefore another quantity is determined: the "Loss Change Index" (LCI) explained as follow.

$$Loss\ Change\ Index = \left( 1 - \frac{cot\theta_{before}}{cot\theta_{after}} \right)$$

By this definition, the Loss Change Index varies between 0 if there is no change in the phase angle and 1 when the phase angle after exposure approach zero and thus the Loss tangent goes infinite.

The loss change index exhibit the following dose-response relation that is about 0.1 at 10 Gy and rise to 0.7 at 20 Gy:

$$y = 1/\ (1 + 10^{(\log\ (17-x)*0.12)})$$

This parameter might be a suitable quantity for assessing the effects of radiation in tissue by impedance measurements.

In conclusion, two parameters seem to help monitor the effect of radiation on tissue, "the ratio $Z_0/R_m$ (Impedance index)" and "Loss Change Index (LCI)". They apply for use at an absorbed dose above 10 Gy per fraction. At lower dose per fraction, they are less accurate and might not be able to use in clinical radiation therapy.

Pirhonen et al. (1995) studied radiation-induced changes in tumour blood flow by colour Doppler ultrasonography. Colour Doppler examination performed on 14 patients with advanced cervical carcinoma treated with external radiotherapy. The total radiation dose varied from 30 to 65 Gy and given as 1.9 Gy daily fractions, 5 days/week: tumour vascularity and blood flow impedance measured by one pre-treatment and five follow-up examinations. At the baseline examination, 11 of 14 patients had very low tumour blood flow impedance (< 0.70). Radiotherapy caused a significant decrease in tumour vascularity (P = 0.0001). The presence of very low blood flow impedance indicates a reduction of tumour vasculature and a better clinical response.

In contrast, the persistence of excessive vascularity or vessels with low blood flow impedance at the end of radiation associate with modest therapeutic response. Eight of 10 patients with increased

tumour vascularity at the end of radiation needed further treatment or died of disease. Only one of four patients with normal vasculature required radiotherapy other treatment. All four were clinically disease-free during the follow-up (mean, 13 months; range, 6-26 months). Their results suggest that colour Doppler ultrasonography helps in the early assessment of therapeutic response during radiotherapy and in planning individual treatment schedules (Pirhonen et al. 1995).

While the ultrasound measurements mainly give information about the blood flow, the impedance measurements information of both blood flow and tissue changes. A correlation between ultrasonography and electrical impedance measurements might investigate further.

**Acknowledgement**

The work financially supported by John and Augusta Persson´s Foundation for scientific and medical research, Lund, and Swedish Cancer Foundation (contract 00 0220)

## REFERENCES

Cole, K. S. & Cole, H. R. 1941, "Dispersion and absorption in dielectrics", *J.Chem.Phys.*, vol. 9, p. 341.

Davidson, D. W. & Cole, H. R. 1950, *J.Chem.Phys.*, vol. 18, p. 1417.

Halle, B., Johannesson, H., & Venu, K. 1998, "Model-free analysis of stretched relaxation dispersions", *Journal of Magnetic Resonance*, vol. 135, no. 1, pp. 1-13.

Nuutinen, J., Lahtinen, T., Turunen, M., Alanen, E., Tenhunen, M., Usenius, T., & Kolle, R. 1998, "A dielectric method for measuring early and late reactions in irradiated human skin", *Radiother Oncol*, vol. 47, no. 3, pp. 249-254.

Osterman, K. S., Paulsen, K. D., & Hoopes, P. J. 1999, "Application of linear circuit models to impedance spectra in irradiated muscle", *Ann N Y Acad Sci*, vol. 873, pp. 21-29.

Pigott, K. H., Dische, S., Vojnovic, B., & Saunders, M. I. 2000, "Sweat gland function as a measure of radiation change", *Radiother Oncol*, vol. 54, no. 1, pp. 79-85.

Pirhonen, J. P., Grenman, S. A., Bredbacka, A. B., Bahado-Singh, R. O., & Salmi, T. A. 1995, "Effects of external radiotherapy on uterine blood flow in patients with advanced cervical carcinoma assessed by color Doppler ultrasonography", *Cancer*, vol. 76, no. 1, pp. 67-71.

Stewart, F. A., Michael, B. D., & Denekamp, J. 1978, "Late radiation damage in the mouse bladder as measured by increased urinary frequency", *Radiation Research*, vol. 75, pp. 649-659.

Stewart, F. A., Lebesque, J. V., & Hart, A. A. M. 1988, "Progressive development of radiation damage in mouse kidneys and consequenses for reirradiation tolerance", *Int.J.Radiat.Oncol.Biol.Phys.*, vol. 53, pp. 405-415.

Turesson, I. & Notter, G. 1986, "The predictive value of skin telangiectasia for late reaction effect in different normal tissues", *Int.J.Radiat.Oncol.Biol.Phys.*, vol. 12, pp. 603-609.

Turesson, I. 1990, "Individual Variation and Dose Dependency in the Progression Rate of Skin Telangiectasia", *International Journal of Radiation Oncology Biology Physics*, vol. 19, no. 6, pp. 1569-1574.

Van der Kogel, A. 1979, *Late effects of radiation on the spinal cord. Dose-effect relationships and pathogenesis* University of Amsterdam.

Bertil_R.Persson@med.lu.se