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Research paper:

Exploration of Electro-Enhanced-Chemotherapy I

Uptake of radioactive tracer in rat Muscle tissue at 6 and 24 hours after applied electric pulses of 600;800;1000;1200 V/cm field-strength, 100;250;500 μs pulse-length, and 2,4,6,8.10.12 number of pulses. (ver.1.0)

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Executive Summary

The aim of the study is to explore the enhanced uptake of the radioactive tracer Technetium-99m-DTPA (^{99m}Tc-DTPA) in rat Muscle tissue in Fischer 344 rats after applied electric pulses of various field-strength, and pulse-length,
Methods: ^{99m}Tc-DTPA (total, 150 MBq) was administered intramuscularly (i.m.) at the shoulder as a bolus in several fractions of 50 μl each in 1 minute intervals. Images of the radioactivity distribution in the rats was recorded with a gamma-camera at 6 and 24 hours after electroporation. EP treatment was performed with 2 needle electrodes separated 8 mm inserted in the right back thigh muscle, through which electric pulses of 600;800;1000;1200 V/cm field-strength, and 100;250;500 μs pulse-length were applied.
Bio-impedance measurements were performed at 2 and 20 kHz through the needle electrodes in the right back thigh muscle. Before applying the EP treatment pulse, two measurements established the reference level R_{before}. Then N_p consecutive pulses (Np = 2,4,6,10,12) of field strength amplitude E = 600.800,1000,1200 V/cm and pulse-length 100,250,500 μs were applied in 1 s interval and the impedance was recorded between each pulse. In order to study the relaxation of the poration the conductance measurements were continued 15 times after the last pulse in 1 s interval.
Statistical analysis and modelling of the data is performed using multivariate data processing methods such as Principal Component Analysis PCA, and modelled with the method of Projection to Latent Structures, PLS, also called PLSR Partial Least Square Regression.
Results: The variation of the uptake ratios with field-strength, pulse-length and number of applied pulses indicate the strongest correlation of is with the number of pulses. This is shown by the outcome of the PLS-modelling equations
$UR6h = 1.709 - 1.32\cdot10^{-3}\times E(V/cm) + 7.67\cdot10^{-3}\times PL(\mu s) + 0.571\times N$ (pulses)
$UR24h = 12.342 - 5.87\cdot10^{-3}\times E(V/cm) - 2.01\cdot10^{-3}\times PL(\mu s) + 0.409\times N$ (pulses)
Conclusion: The most optimal scenario to predict the outcome of the electro-enhanced chemotherapy session i.e. to achieve highest uptake ratio of Bleomycin would be to use LCI 20kHz; LCI 2kHz as descriptor beside the parameters E, PL an N.

1. Introduction

The principles for application of high voltage impulses in vivo for tumour therapy and gene therapy has previously been described in detail (Persson 2000). The aim of the present study is to explore the enhanced uptake of the radioactive tracer Technetium-99m-DTPA (^{99m}Tc-DTPA) in rat Muscle tissue in Fischer 344 rats after applied electric pulses of 1000 V/cm field-strength, 100μs pulse-length, and various number of pulses.

2. Exploration of Radioactivity Uptake

Animals

Healthy Fischer-344 rats (B&K; Stockholm, Sweden) and Wistar rats (Taconic M&B; Ry, Denmark) were used in the experiments. The animals were housed in polycarbonate cages with access to food and fresh water *ad libitum*. Both male and female rats were used, weighing 300–400 and 150–200 g, respectively. Before electric pulse treatment, the animals were anesthetized with either chloral hydrate or isoflurane (Forene; Abbott Scandinavia AB, Solna, Sweden) in by applying "Univentor 400 anaesthesia unit."

The Animal Ethical Committee in Malmö/Lund (Permit M171-04; Lund, Sweden) approved all experimental animal procedures.

Radiopharmaceutical

Technetium-99m (^{99m}Tc) is a radioisotope with physical characteristics suitable for *in vivo* tracer experiments. It has a half-life of 6.0 hours and emits gamma photons of 140 keV (87% per decay), which results in a low absorbed dose per activity unit (Bq) and high detection efficiency in thin NaI(Tl) crystals used in gamma cameras.

The radiopharmaceutical ^{99m}Tc-DTPA is a stable, water-soluble compound (MW 416) used clinically in radionuclide angiography, static brain imaging, and kidney and urinary tract studies. ^{99m}Tc-DTPA was chosen as the tracer in this study because its pharmacokinetic behaviour is very similar to bleomycin (MW 1400).

^{99m}Tc-DTPA is prepared from a kit of TechneScan® (Mallinckrodt Medical B.V.; Petten, Holland). This kit is a freeze-dried sterile mixture of 25 mg Ca-Na-3-diethylene-triamine-pentaacetate (DTPA), 0.21 mg stannous-chloride-dihydrate ($SnCl_2 \cdot H_2O$), 0.25 g Gentisic acid that is a di-hydroxy-benzoic acid, used as an antioxidant excipient, and 12 mg sodium chloride. By adding 300 MBq ^{99m}Tc-sodiumpertechnetate in 0.75 mL of sterile, pyrogen-free physiological saline, mixed until the powder is dissolved, ^{99m}Tc-DTPA is formed. After 15 minutes at room temperature, the ^{99m}Tc-DTPA solution is ready for injection and is stable for 8 hours. The labelled compound is a slightly opalescent and colourless aqueous solution with a pH of 4.0–5.0, with a labelling efficiency 95%. In the present study the ^{99m}Tc-DTPA (total, 150 MBq) was administered intramuscularly (i.m.) at the shoulder as a bolus in several fractions of 50 µl each in 1 minute intervals.

Radioactivity measurement

Images of the radioactivity distribution in a typical rat under the gamma-camera (GK) is shown in **Figure 1** at 6 hours after electric pulse exposure and in **Figure 2** after 24 hours. The administration site at the shoulder appear as a dark spot and the uptake in electric pulse treated region at the thigh is seen as a dark spot. The corresponding area at the opposite untreated side is used as reference for extracellular activity. Kidney(K), bladder(B) with urine activity are seen as dark areas in the picture.

In the evaluation of the GK-images shown in **Figure 1**, a line profile was placed across the treated muscle and "*Full Width at Half Maximum*" (FWHM) of the electroporated region was determined for the gamma camera recorded activity peak. Line profile of the recorded pulse height distribution was

determined for this profile. This measure define the diameter of the circular Region of Interest (ROI) and used for the evaluation of the drug uptake-ratio in all animals treated in that particular series.

The present study investigate the effect of applied electric pulses of 600; 800; 1000: 1200: V/cm 100 and 500 µs pulse length, and 2, 4, 6 or 12 pulses, on the accumulation of the radiolabelled pharmaceutical ^{99m}Tc-DTPA that mimics the Bleomycin, in rat muscular tissue after *in vivo* electropermeabilization. Gamma camera measurements is applied to noninvasively quantify the accumulation of ^{99m}Tc-DTPA in the region treated with electrical pulses as previously described.

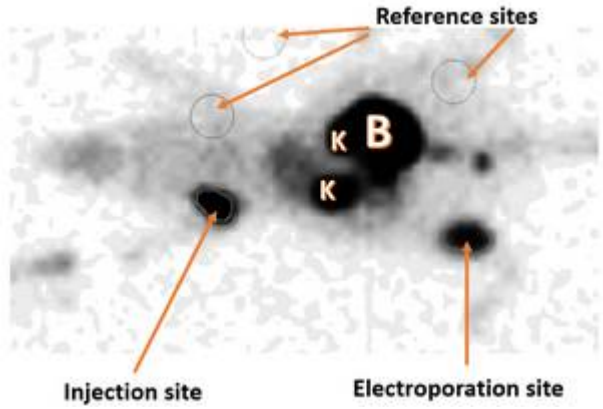

Figure 1a
GK-image of a rat at 6 h after electroporation treatment with 800 V/m; 0.25 ms; 12 pulses; K= kidney, B= bladder

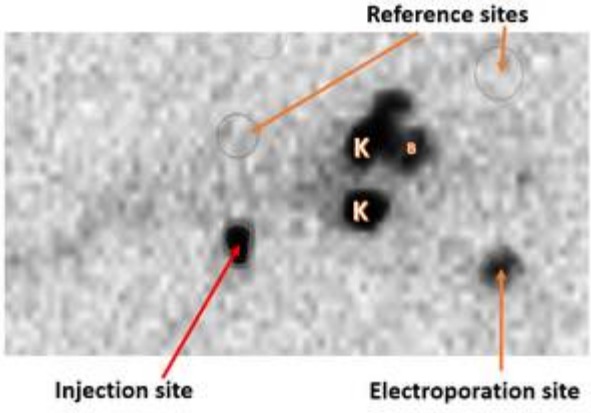

Figure 1b
GK-image of the same rat as in 1a at 24 h after electroporation treatment with 800 V/m; 0.25 ms 12 pulses. K= kidney, B= bladder

In **Table 1** is displayed for each rat: the applied field strength (V/cm), pulse-length (µs), number of applied pulses N, and the uptake-ratio of ^{99}Tc^{m}-DTPA in the target region after 6 h (UR6h) and after 24 hours (UR24h).

Table 1 Electroporation variables and results of Activity Uptake measurements

Rat ID	E V/cm	PL µs	N pulses	TcUR6h	±Sd	TcUR24 h	±Sd
1001	1000	100	6	1,45	0,07	1,61	0,36
1002	1000	100	6	3,58	0,16	4,44	0,39
1003	1000	100	6	3,26	0,16	11,59	4,27
1004	1000	100	12	9,72	0,56	5,77	0,83
1005	1000	100	12	10,63	0,72	12,84	1,23
1006	1000	100	12	6,66	0,40	11,99	2,79
1007	1000	500	12	10,72	0,53	18,13	3,20
1008	1000	500	12	14,52	0,72	6,74	0,68
1009	1000	500	12	10,14	0,37	13,05	1,49
81	800	250	12	12,93	0,51	17,23	2,61
82	800	250	12	11,10	0,59	18,90	1,67
83	800	250	12	12,28	0,66	21,78	4,76
61	600	500	12	9,21	0,36	7,13	0,60
62	600	500	12	5,76	0,22	5,88	1,30

Continued

Table1 continued

Rat ID	E V/cm	PL µs	N pulses	TcUR6h	±Sd	TcUR24 h	±Sd
84	800	500	12	13,31	0,70	12,11	1,79
85	800	500	12	11,20	0,59	9,32	0,83
86	800	500	12	10,25	0,42	9,30	1,05
1218	1200	500	12	12,84	0,56	14,17	1,51
1219	1200	500	12	11,23	0,50	5,61	0,53
1220	1200	500	12	7,59	0,30	8,05	1,14
1215	1200	500	10	8,43	0,30	8,01	0,77
1216	1200	500	10	8,03	0,30	6,48	0,77
1217	1200	500	10	12,45	0,59	5,82	0,59
1212	1200	500	8	9,01	0,33	9,07	0,82
1213	1200	500	8	5,81	0,20	4,70	0,48
1214	1200	500	8	5,53	0,22	4,22	0,46
1209	1200	500	6	8,92	0,39	7,15	0,65
1210	1200	500	6	4,41	0,18	2,79	0,18
1211	1200	500	6	7,73	0,56	4,12	0,52
1204	1200	500	4	10,35	0,48	8,36	0,92
1205	1200	500	4	7,57	0,38	8,93	1,40
1206	1200	500	4	13,49	0,70	14,81	1,99
1207	1200	500	4	7,59	0,34	17,49	1,34
1208	1200	500	4	6,18	0,33	3,02	0,38
1201	1200	500	2	3,34	0,18	3,22	0,56
1202	1200	500	2	2,29	0,13	1,53	0,25
1203	1200	500	2	2,92	0,14	2,52	0,41

Statistical analysis and modelling of the data is performed using multivariate data processing methods such as Principal Component Analysis PCA, and modelled with the method of Projection to Latent Structures, PLS, also called PLSR Partial Least Square Regression. Herman Wold introduced the method of Partial least squares (Wold, 1982). His son Svante Wold, who was a chemist has then developed the method to be used in chemometrics, and according to him, the *projection to latent structures* should be the correct name of the method (Wold et al., 2001). These methods are nowadays commonly used in chemometrics,_bio-pharmacology and related areas. *Principal component analyses* PCA and *clustering* are used to study the quality and structure of the original database. PCA can also be used to find outliers and to find out if the data can be divided into various classes. In order to find an equation to predict the dependent variables from the descriptors, the model of *Projection to Latent Structure regression* (PLSR) was used (XLSTAT, 2015).

Table 2.

Correlation matrix with E, PL and N as descriptor variables, with UR6h and UR24 as depending variables.

Variables	E V/cm	PL µs	N pulses	UR6h	UR24h
E V/cm	**1,000**	0,305	-0,591	-0,252	-0,356
PL µs	0,305	**1,000**	-0,163	0,172	-0,197
N pulses	-0,591	-0,163	**1,000**	0,616	0,434
UR6h	-0,252	0,172	0,616	**1,000**	0,612
UR24h	-0,356	-0,197	0,434	0,612	**1,000**

The strongest correlation of the uptake rations is with the number of pulses. This is clearly demonstrated in **Figure 2** with the plot of the correlation of the variables with PCA score values. The display indicate that the $^{99r}Tc^{m}$ Uptake ratio is close connected to the number of pulses applied in the session, but independent of pulse-length and inverse correlated to the applied pulse length. This is confirmed by the outcome of the modelling equations given below.

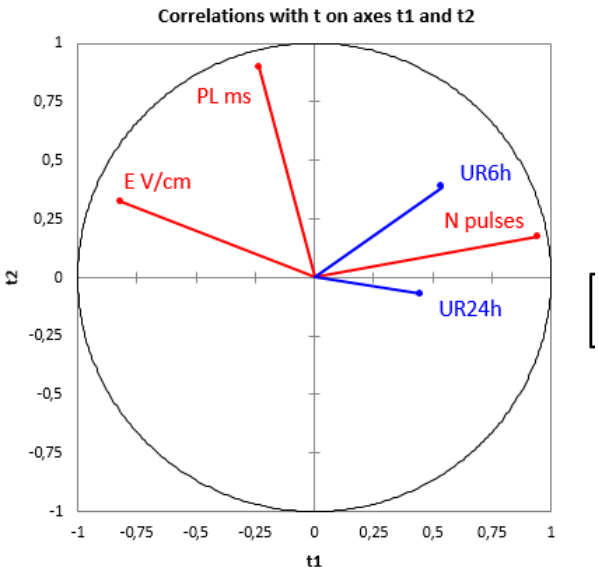

Figure 2
Plot of the Correlation of the variables with the t1 and t2 PCA components.

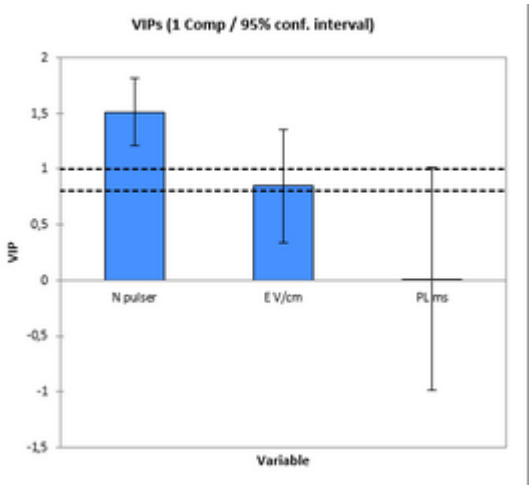

Figure 3
The ***variable importance of projections*** **(VIP)** of the experimental variables used in the modelling of Projection of latent variables.

The variable importance of projections (VIP) of the descriptor variables used in the modelling of Projection of latent variables are displayed in **Figure 3**

The linear relations after PLS modelling of the uptake ratios at 6 and 24 hours after the electroporation with corresponding parameters.

Table 3
Model parameters:

Variable	UR6h	UR24h
Intercept	1,709	12,342
E V/cm	-1,32·10^{-3}	-5,87·10^{-3}
PL µs	7,67·10^{-3}	-2,01·10^{-3}
N pulses	0,571	0,409
R^2	0.44	0.21

Equations of the model:

UR6h = 1,709 - 1.32·10^{-3}×E(V/cm) + 7.67·10^{-3} ×PL(µs) + **0.571**×**N (pulses)**

UR24h = 12,342 – 5.87·10^{-3}×E(V/cm) – 2,01·10^{-3}×PL(µs) + **0,409**×**N (pulses)**

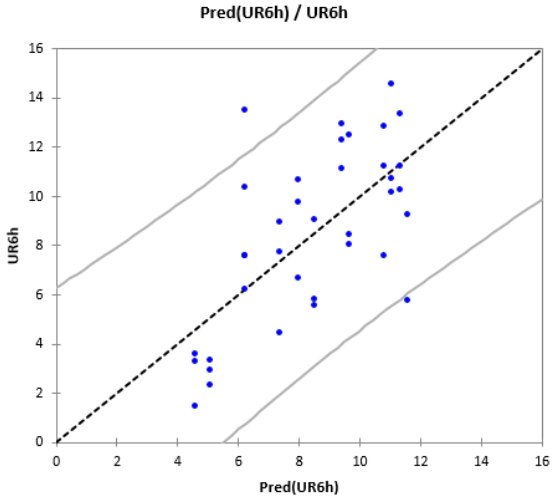

Figure 4a
The regression between the experimental and predicted uptake ratios at 6 h after EP

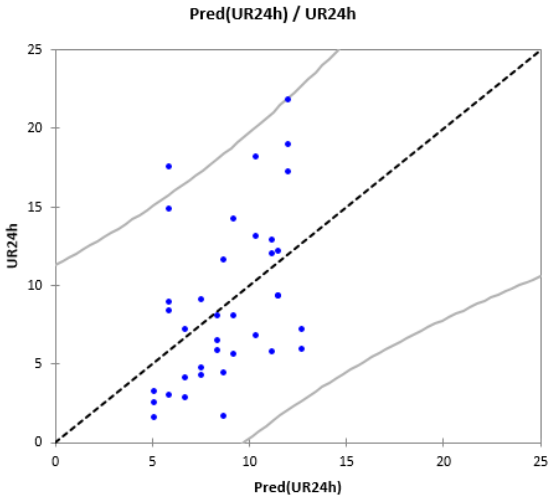

Figure 4b
The regression between the experimental and predicted uptake ratios at 24 h after

3. Exploration of conductivity change and loss change index

Conductance measurements

Resistivity measurements were performed at 2 and 20 kHz in Fischer 344 rats with 2 needle electrodes separated 8 mm inserted in the right back thigh muscle. Before applying the EP treatment pulse, two measurements established the reference level R_{before}. Then N_p consecutive pulses (Np = 2, 4, 6, 10, 12) of field strength amplitude E = 600.800,1000,1200 V/cm and pulse-length 100,250,500 µs were applied in 1 s interval and the impedance was recorded between each pulse. In order to study the relaxation of the poration the conductance measurements were continued 15 times after the last pulse in 1 s interval.

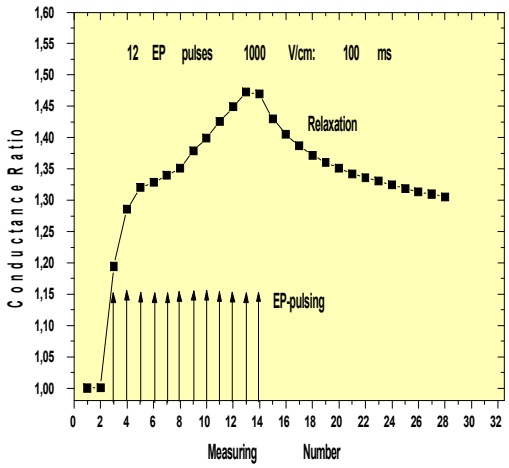

Figure 5
Conductance ratio before, during and after 12 consecutive EP pulses of 1000 V/cm amplitude and 100 μs pulse length.

In **Figure 5** are displayed as an example, the results of relative conductance G_{rel}

$$G_{rel} = G_{after} / G_{before} = R_{before} / R_{after}$$

after each pulse of electroporation with 1000 V/cm and pulse-length 100 μs and each second up to 15 s thereafter.

Resistivity change index, RCI

The resistivity change index is defined as the relative change in resistivity after electroporation

$$RCI = \left(1 - \frac{R_{after}}{R_{before}}\right)$$

$\mathbf{R_{before}}$ is the resistivity (Ω) measured before the first pulse.
$\mathbf{R_{aftet}}$ is the resistivity after electroporation with N_p **pulses**

Loss Change Index LCI

Loss change index is defined as the relative change in the tangent for phase angle ϕ after electroporation

$$LCI = (1\text{-}\tan\phi_{after}/\tan\phi_{before})$$

Measurements of the phase angle ϕ were performed at 2 and 20 kHz in Fischer 344 rats with 2 needle electrodes separated 8 mm inserted in the right back thigh muscle. Before applying the EP treatment pulse, two measurements established the reference level $\tan\phi_{before}$. Then N_p consecutive pulses (Np = 2,4,6,10,12) of field strength amplitude E = 600.800,1000,1200 V/cm and pulse-length 100,250,500 μs

Projection of Latent variable modelling was performed with RCI and LCI at dependent variables and field strength amplitude E, pulse Length PL and number of pulses Np as descriptor variables as shown in Table 4. The rat no.15 and 18 was found of to be outliers according to Grubbs test.

Table 4

Resitivity change index, RCI, and Loss Change Index LCI recorded at 2 and 30 kHz with E, PL and N as predictor variables.

Rat ID	E V/cm	PL µs	N pulser	RCI2kHz	RCI20kHz	LCI2kHz	LCI20kHz
1	1200	500	2	0,325538	0,289199	0,365323	0,209785
2	1200	500	2	0,385064	0,345726	0,348878	0,116239
3	1200	500	2	0,372426	0,326972	0,331704	0,193031
4	1200	500	4	0,370256	0,271888	0,432566	0,379131
5	1200	500	4	0,539428	0,465996	0,428167	0,435437
6	1200	500	4	0,504138	0,423764	0,54272	0,379131
7	1200	500	4	0,452006	0,368696	0,437921	0,351507
8	1200	500	4	0,420968	0,355364	0,403846	0,286832
9	1200	500	4	0,695404	0,658222	0,479897	0,351507
10	1200	500	10	0,43832	0,329494	0,513916	0,554743
11	1200	500	10	0,531103	0,461318	0,494895	0,494702
12	1200	500	10	0,613523	0,567744	0,457572	0,497419
13	1200	500	12	0,724213	0,667551	0,601524	0,618027
14	1200	500	12	0,769949	0,721743	0,548343	0,604093
16	1000	100	6	0,425754	0,333345	0,461687	0,379131
17	1000	100	6	0,228959	0,167615	0,348999	0,209785
19	1000	100	12	0,319345	0,251516	0,343155	0,347313
20	1000	100	12	0,423074	0,365181	0,399485	0,312056
21	1000	100	12	0,499393	0,424577	0,406254	0,483461
22	1000	500	12	0,631195	0,576461	0,452555	0,512543
23	1000	500	12	0,474665	0,377899	0,495281	0,56007
24	1000	500	12	0,557485	0,469206	0,503889	0,633995
25	800	500	12	0,432579	0,343002	0,563409	0,454837
26	800	500	12	0,485166	0,400648	0,546538	0,56007
27	800	500	12	0,572739	0,497544	0,525134	0,559179
28	800	250	12	0,361432	0,305608	0,391293	0,286832
29	800	250	12	0,620463	0,568178	0,440384	0,483461
30	800	250	12	0,523003	0,486987	0,331697	0,483461
31	600	500	12	0,397096	0,324021	0,460259	0,379131
32	600	500	12	0,372689	0,29248	0,411118	0,435437
Outliers							
15	1200	500	12	0,968177	0,934328	0,620074	0,91418
18	1000	100	6	0,519251	0,50865	0,32102	0,069188

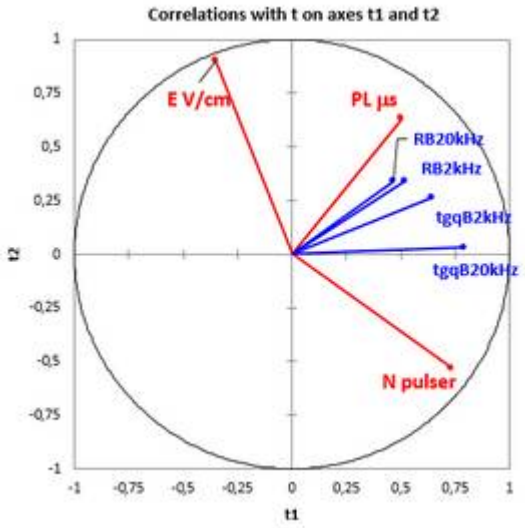

Figure 6a
Plot of the Correlation the first and second principal latent component of the various variables.

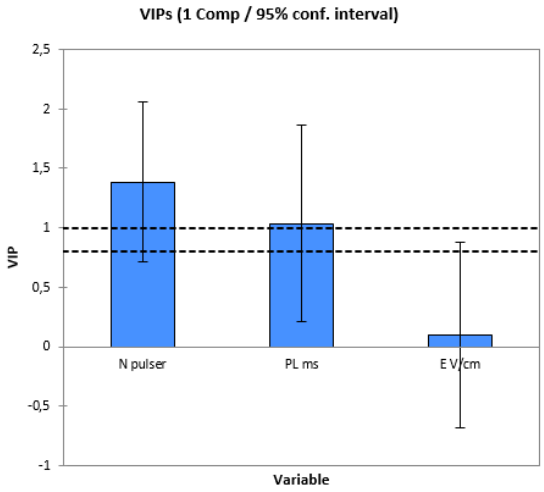

Figure 6b
Variable Importance in the 1st Projection (VIP) with the largest variance

Figure 6a indicate that the resistivity and phase variables are closely correlated to the pulse length and number of applied pulses. As shown in **Figure 6b** the most *important variable in the projection* (VIP) is the number of pulses closely followed by the pulse length the applied electric field seems to be less important.

Table 5
Model parameters of PLS modelling

Variable	RCI2kHz	RCI20kHz	LCI2kHz	LCI20kHz
Intercept	-0,226	-0,288	0,114	-0,280
E V/cm	3,76E-04	3,85E-04	1,20E-04	2,65E-04
PL µs	2,75E-04	2,57E-04	2,49E-04	3,03E-04
N pulses	0,024	0,023	0,012	0,034
R^2	0,453	0,392	0,491	0,723

The best goodness of fit value R^2 vas found for LCI recorded at 20 kHz for which the *following equations of the model were achieved:*

Loss Change Index LCI 20 kHz:

$$\mathbf{LCI20kHz = -0.280 + 2.65\cdot10^{-4}\times E\ V/cm + 3.03\cdot10^{-4}\times PL\mu s + 0.034\times N}$$

4. Exploration of Current density J and Specific Absorbed Energy W

The current density J A/cm^2 is estimated by the equation J=σ·E=S/m·V/m=A/m^2

$$J = \sigma \cdot E$$

While the current I = J·(area m^2) A . The cross section area of current between the needle electrodes is estimated to 1 cm^2=0.01 m^2

The specific absorbed energy W is calculated from the following expression

$$W = \sum_p \frac{\sigma_p \cdot E^2}{\rho} \cdot t_p \cdot N_p \quad [J.kg^{-1}]$$

where

- σ is the tissue conductivity for the tissue [S/m]
- E is the electric field strength [V/m]
- t_p is the pulse length [s]
- N_p is the number of applied pulses
- ρ is the density of tissue (muscle 1060 $kg.m^{-3}$)

The conductivity of the tissue after electroporation is predicted by the equation

$$\sigma_{after} = \sigma_{before} \cdot R_{before}/R_{after}$$

Table 4

The current density J A/cm^2 and specific absorbed energy W $J.kg^{-1}$ with E, PL and N as predictor variables

Rat ID	E V/cm	PL µs	N pulses	J $A.cm^{-2}$	W $J.kg^{-1}$
1	1200	500	2	14,47	3276
2	1200	500	2	16,17	3660
3	1200	500	2	15,32	3547
4	1200	500	4	15,50	7018
5	1200	500	4	25,00	11321
6	1200	500	4	20,69	9370
7	1200	500	4	16,89	7647
8	1200	500	4	17,07	7732
9	1200	500	4	29,38	13306
10	1200	500	10	15,67	17735
11	1200	500	10	18,95	21448
12	1200	500	10	23,29	26364
13	1200	500	12	32,19	43725
14	1200	500	12	37,57	51039
16	1000	100	6	2,74	1549
17	1000	100	6	2,22	1256
18	1000	100	6	2,34	1326
19	1000	100	12	2,32	2632
20	1000	100	12	2,63	2983
21	1000	100	12	3,12	3529
22	1000	500	12	3,34	21165
23	1000	500	12	3,82	15574
24	1000	500	12	3,16	17881

Continued

Table 4 continued					
25	800	500	12	2,15	9754
26	800	500	12	2,28	10303
28	800	250	12	1,91	4327
29	800	250	12	3,11	7034
30	800	250	12	2,66	6029
31	600	500	12	1,64	5580
32	600	500	12	1,44	4899
Outlier					
15	1200	500	12	258,15	45482

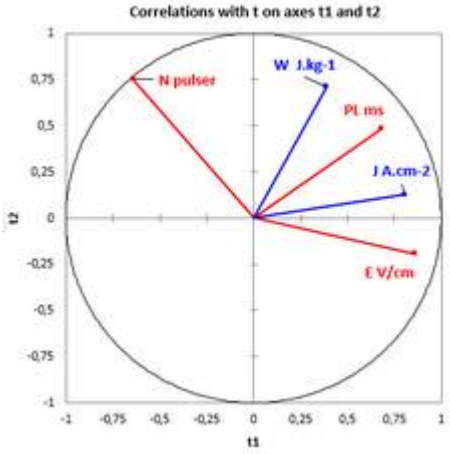

Figure 7a
Plot of the Correlation the first and second principal latent component of the various variables.

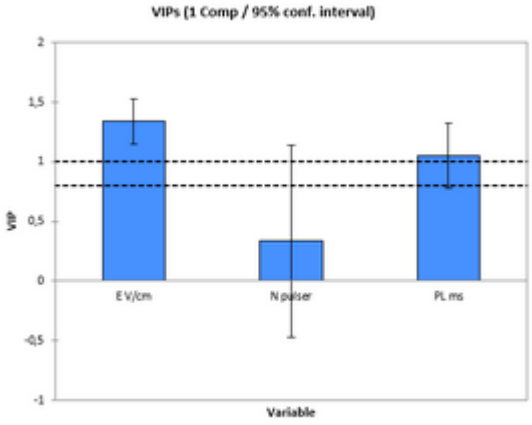

Figure 7b
Variable Importance in the 1st Projection (VIP) with the largest variance

Projection of Latent variable modelling was performed with J and W as dependent variables and field strength amplitude E, pulse Length PL and number of pulses Np as descriptor variables as shown in Table 5. The rat no. 15 was found of to be outliers according to Grubbs test.

Table 5
Model parameters of PLS modelling

Variable	J A.cm^{-2}	W J.kg^{-1}
		-
Intercept	-45,171	71790,643
E V/cm	0,043	47,846
PL µs	0,022	28,579
N pulses	0,398	2615,750
R^2	0,705	0,740

The best goodness of fit value R^2 was found for LCI recorded at 20 kHz for which the *following equations of the model were achieved:*

With current density and Specific Absorbed Energy as dependent variables and applied field strength, pulse length and number of pulses as descriptor variables a PLSR modelling was performed. The following model equations were achieved:

$$\mathbf{J\ A.cm^{-2} = -\ 45.2 + 0.043 \times E\ V/cm + 0.022 \times PL\ \mu s + 0.398 \times N\ pulses}$$
$$\mathbf{W\ \ J.kg^{-1} = -\ 71791 + 47.9 \times E\ V/cm + 28{,}6 \times PL\ \mu s + 2616 \times N\ pulses}$$

5. Exploration of relaxation and fraction of reversible electroporation

After the applied electro-permeabilization, pulse the conductivity start to decrease and approach a plateau value. The fraction of the plateau value relative to the conductivity recorded after the last pulse is a measure of the fraction of reversible electropermeabilized cells. This value is of importance for the long term transfer of exogenous substances to the cell and outflow of immunogenic substances from the cell. The relaxation curves for each rat was fitted to a single exponential decay

$$\sigma_{rlp} = A_0 + f_{rev} \cdot exp(-t/T1)$$

where

f_{rev} is the fraction of reversible electroporation
$A_0 = 1 - f_{rev}$ is the fraction of irreversible electroporation

At t=0 $\sigma_{rlp} = A_0 + f_{rev} = 1$

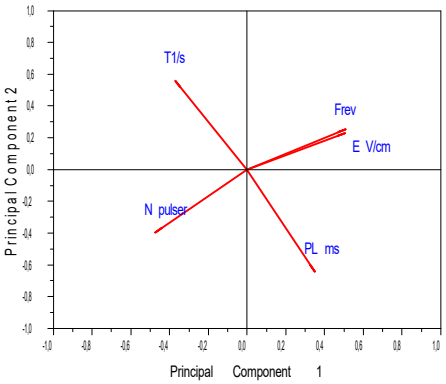

Figure 8a
PCA analysis

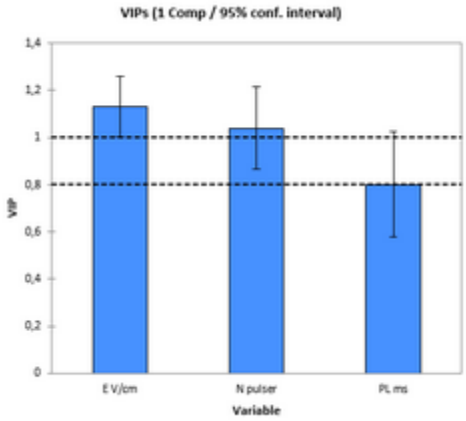

Figure 8b
Variable Importance in the Projection (VIP):

Table 6
Model parameters of PLS modelling

Variable	T1/s	f_{rev}
Intercept	6,056	-0,257
E V/cm	-1,82E-03	5,07E-04
PL µs	-1,18E-03	3,28E-04
N pulses	0,065	-0,018
R^2	0,295	0,725

6. Exploration of radioactivity uptake with all descriptors

PLS analysis of the activity ratios at 6 and 24 hours as depending variables and J A.cm^{-2}, W J.kg^{-1}, RCI 2kHz, RCI 20kHz, LCI 2kHz, LCI 20kHz, T1/s, f_{rev} as descriptor variables Showed that the variables of most important projection were LCI 20 kHz followed by LCI 2 kHz, W J.kg-1, RCI 2 kHz. Low VIP values showed<1 showed RCI 2kHx, f_{rev}, T1 and J.

Table 7

Variable Importance in the Projection (VIP): for the investigated variables and the Pearson's regression coefficient for the correlation between the Activity uptake ratios and the corresponding variable

Variable	VIP	Standard deviation	Pearson's R pred 6h	Pearson's R meas. 6h	Pearson's R pred 24h	Pearson's R meas. 24h
tgqB20kHz	1,738	0,274	0.91	0.51	0.91	0.24
tgqB2kHz	1,134	0,444	1	0.4	0.85	-0.01
W J.kg-1	1,063	0,267	0.85	0.55	0.85	0.17
RB2kHz	1,055	0,318	0,84	0.36	0,84	0,33
RB20kHz	0,898	0,391	0.77	0.30	0.77	0.33
Frev	0,638	0,570	-0.35	-0.18	-0.34	-0.14
T1/s	0,411	0,316	-10^{-4}	-0.007	-10^{-4}	0.24
J A.cm-2	0,263	0,263	0.22	0.04	0.22	-0.22

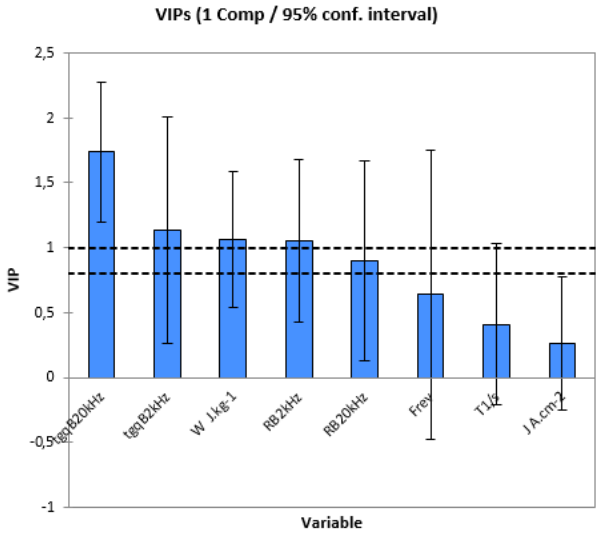

Figure 9

Variable Importance in the Projection (VIP):

Regression of the various impedance descriptors with the predicted and measured values of $^{99}Tc^{m}$ uptake ratios.

Table 8
Model parameters of PLS modelling

:

Model parameters Variable	UR6h	UR24h
Intercept	4,056	6,446
J A.cm-2	-0,002	-0,001
W J.kg-1	0,000	0,000
RCI 2kHz	1,954	1,291
RCI 20kHz	1,573	1,040
LCI 2kHz	3,855	2,547
LCI 20kHz	2,834	1,873
T1/s	0,004	0,003
f_{rev}	-0,920	-0,608

In the following section the predicted and recorded Tc uptake ratios are correlated with the various descriptors one by on in follow in order of importance (VIP value)

Loss Change index measured at 20 kHz

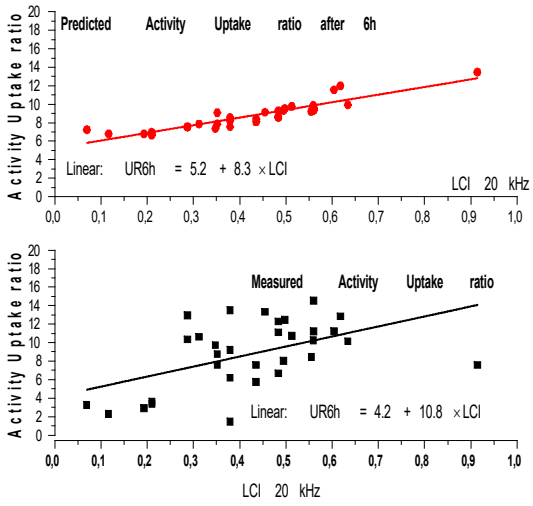

Figure 10a
Uptake ratio after 6 h versus LCI recorded at 20 kHz
Pearson's R: 0.91(pred.); 0.51(meas.)

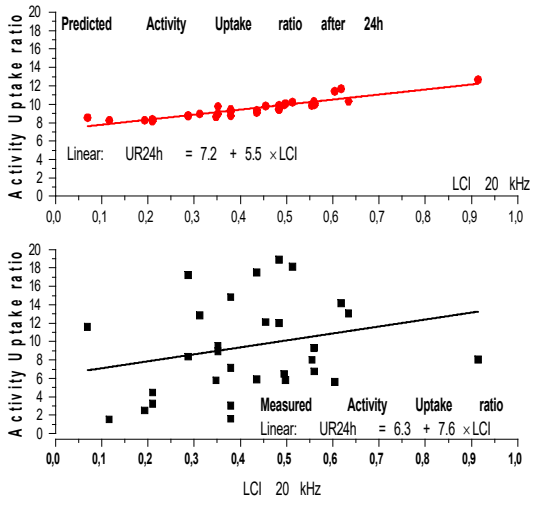

Figure 10b
Uptake ratio after 24 h versus LCI recorded at 20 kHz
Pearson's R: 0.91(pred.); 0.24(meas.)

Loss Change index measured at 2 kHz

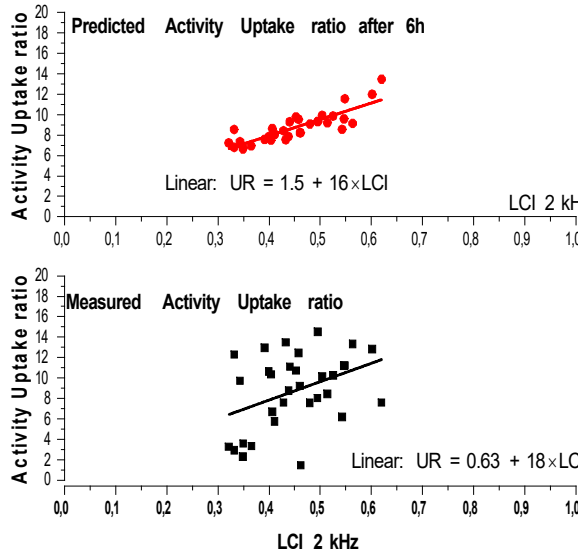

Figure 11a Uptake ratio after 6 h versus LCI recorded at 2 kHz Pearson's R: 1(pred.); 0.4(meas.)

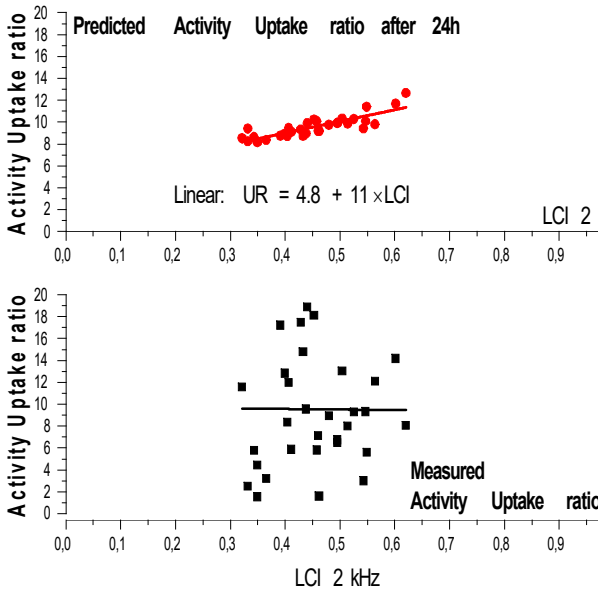

Figure 11b Uptake ratio after 24 h versus LCI recorded at 2 kHz Pearson's R: 0.85 (pred.); -0.01(meas.)

Specific Absorbed energy W J.kg^{-1}

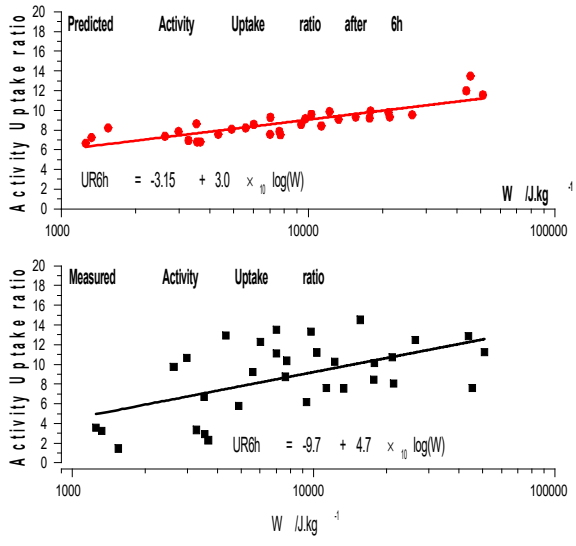

Figure 12a Uptake ratio after 6 h versus Specific absorbed energy W (J.kg^{-1}) Pearson's R: 0.85(pred.); 0.55(meas.)

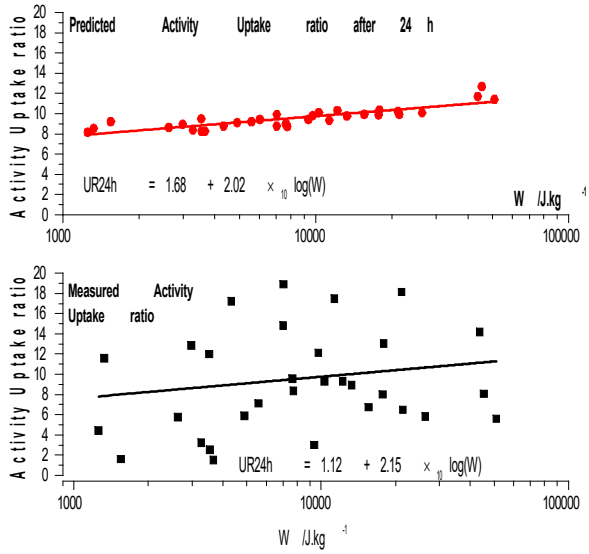

Figure 12b Uptake ratio after24 h versus Specific absorbed energy W (J.kg^{-1}) Pearson's R: 0.85(pred.); 0.17(meas.)

LCI 2kHz

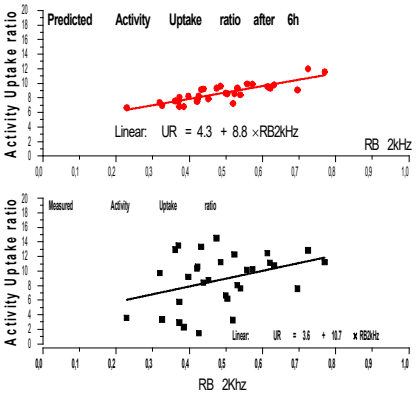

Figure 13a Uptake ratio after 6 h versus RB2kHz
Pearson's R: 0,84 (pred.); (meas.) 0.36

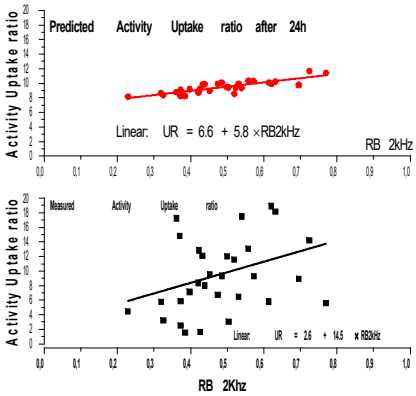

Figure 13b Uptake ratio after 24 h versus RB2kHz
Pearson's R: 0.84 (pred.); 0,33 (meas.)

RCI 20kHz

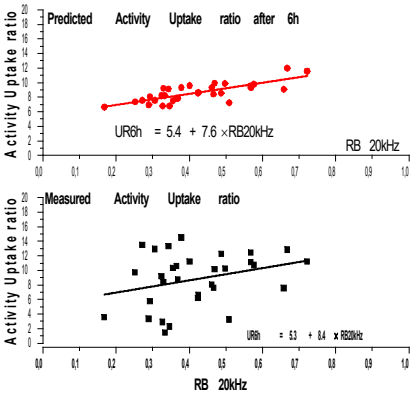

Figure 14a Uptake ratio after 6 h versus RB20kHz
Pearson's R: 0.77 (pred.); 0.30 (meas.)

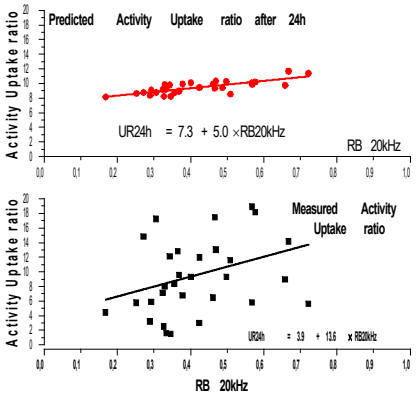

Figure 14b Uptake ratio after 24 h versus RB20kHz
Pearson's R: 0.77 (pred.); 0.33(meas.)

Fraction Reversible, f_{rev}

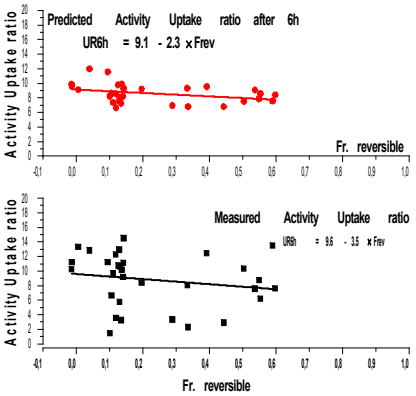

Figure 15a Uptake ratio after 6 h versus Frev
Pearson's R: -0,35 (pred.); -0.18 (meas.)

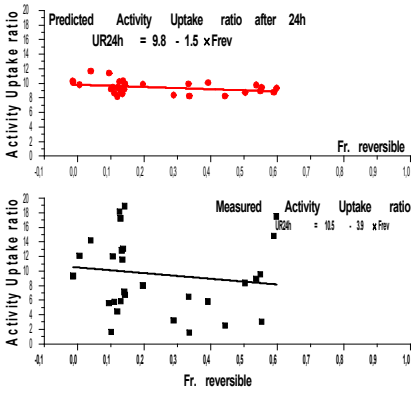

Figure 15b Uptake ratio after 24 h versus Frev
Pearson's R: -0,34 (pred.); -0,14 (meas.)

Relaxation time T1 /s

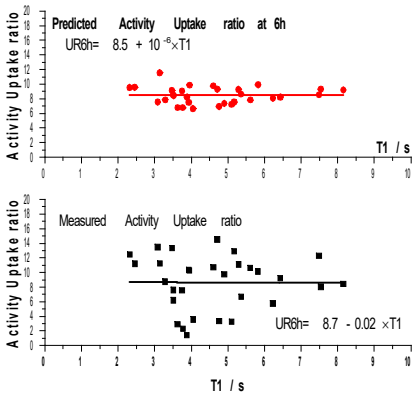

Figure 16a Uptake ratio after 6 h versus T1 Pearson's R: -10^{-4} (pred.); -0,007 (meas.)

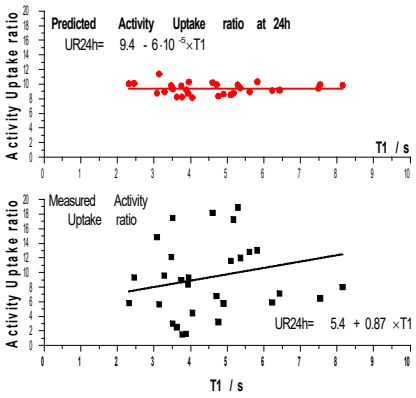

Figure 16b Uptake ratio after 24 h versus T1 Pearson's R: -10^{-4} (pred.); 0.24 (meas.)

Current density J A.cm^{-2}

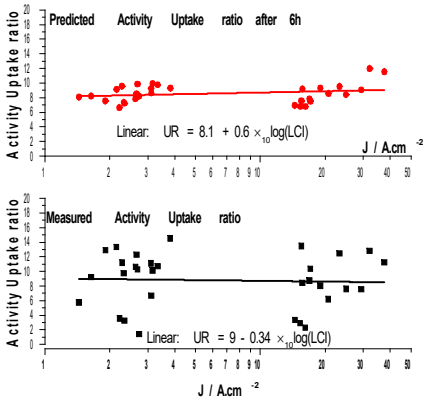

Figure 17a Uptake ratio after 6 h versus current density J(A.cm^{-2}) Pearson's R: 0.22(pred.); 0.04(me .)

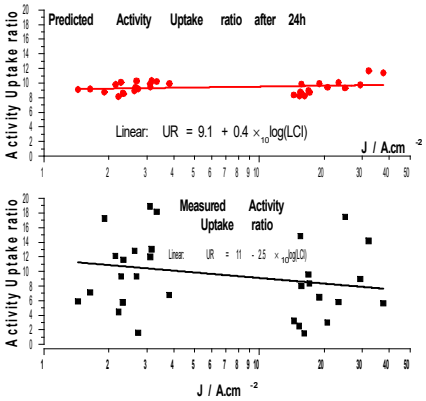

Figure 17b Uptake ratio after 24 h versus current density J(A.cm^{-2}) Pearson's R: 0.22(pred.); -0.22(meas.)

7. Conclusion

The parameters with best regression to the uptake ratio at 6 and 24 hours after electroporation are LCI 20kHz; LCI 2kHz; W J.kg^{-1}; RCI 2kHz with significant high Pearson's regression coefficient. The correlation coefficient ranges from −1 to 1. A value of 1 implies that a linear equation describes the relationship between X and Y perfectly, with all data points lying on a line for which Y increases as X increases. A value of −1 implies that all data points lie on a line for which Y decreases as X increases. A value of 0 implies that there is no linear correlation between the variables.

Thus the most optimal scenario to predict the outcome of the electro-enhanced chemotherapy session i.e.to achieve highest uptake ratio of bleomycin would be to use LCI 20kHz; LCI 2kHz as descriptor beside the parameters E, PL an N.

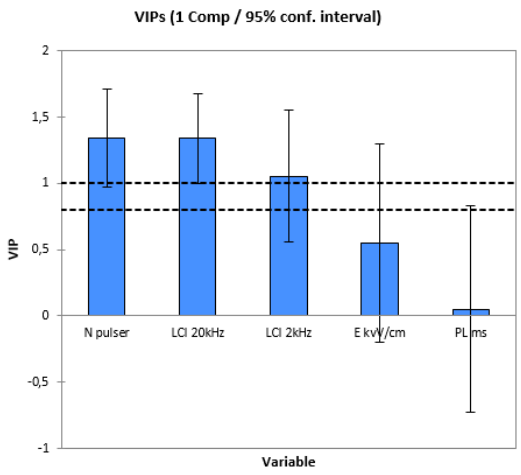

Figure 18a Variable Importance in the Projection (VIP):

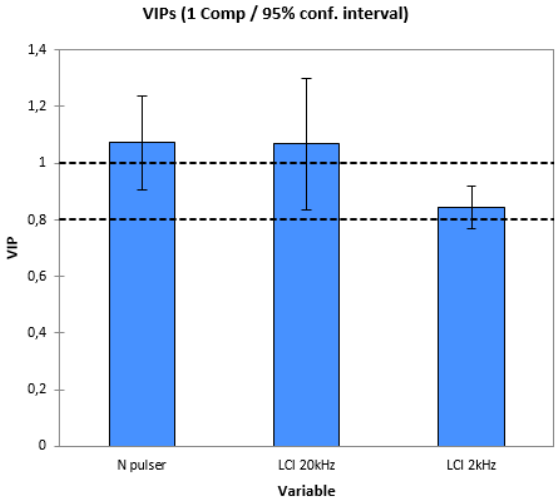

Figure 17b Variable Importance in the Projection (VIP):

Modelling with all five descriptors LCI 20kHz; LCI 2kHz, E kV/cm, PL µs, and N) resulted in the coefficients of Table 9 .

Surprisingly the coefficient for the E-field become negative that means that it is detrimental for the uptake to increase the field strength. It also indicates that the homogeneity of the field is of less importance.

Table 9
Model parameters of PLS modelling

Variable	UR6h	UR24h
Intercept	2,22	3,37
E kV/cm	-1,85	-1,61
PL µs	-0,22	-0,19
N pulses	0,22	0,19
LCI 2kHz	9,38	8,13
LCI 20kHz	6,32	5,48
R^2	0,46	0,16

Equations of the model:

UR6h = 2.22 - 1.85×E kV/cm - 0.22×PL µs + 0.22×N pulses + 9.38×LCI 2kHz +6.32xLCI 20kHz

By omitting the E-field and pulse length as descriptor variables the following modelling results was achieved results

Table 10
Model parameters of PLS modelling

Variable	UR6h	UR24h
Intercept	-0,51	1,46
N pulses	0,24	0,20
LCI 2kHz	10,17	8,34
LCI 20kHz	6,82	5,60
R^2	0,47	0,14

UR6h = -0.51 + 0.24×N pulses+ 10.17×LCI 2kHz +6.82xLCI 20kHz

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