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● *Biology Contribution*

PULSED BRACHYTHERAPY AS A SUBSTITUTE FOR CONTINUOUS LOW DOSE RATE: AN *IN VITRO* STUDY WITH HUMAN CARCINOMA CELLS

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Purpose: Pulsed dose rate (PDR) brachytherapy as a substitute for continuous low dose rate (CLDR) has the potential to be a useful option in brachytherapy. However, the frequency and duration of pulses that will produce results practically equivalent to CLDR is still an open and important question. This study was designed to compare the survival of human tumor cells, cultured *in vitro*, and exposed to continuous or pulsed irradiation where the pulse frequency was varied.

Methods and Materials: Three different human carcinoma cells, derived from cervical and breast cancers, were exposed to CLDR gamma rays, or to pulsed irradiations with the same overall dose rate. Pulsed regimens used were 3.8 min every hour, 7.6 min every 2 h, 11.4 min every 3 h, 15.2 min every 4 h, 22.8 min every 6 h, and 45.6 min every 12 h. For each comparison between CLDR and PDR, the overall dose and the overall time were the same. Experimental design was such that significant differences in biological effectiveness, if present, would be detected.

Results: For the cell lines investigated, hourly pulses resulted in cell survival indistinguishable from CLDR. However, as the pulse interval was increased, cell survival progressively decreased compared with CLDR, and the pulsed regimes were no longer equivalent to continuous low dose rate.

Conclusion: This study provides some evidence to support the suggestion that a 10-min pulse, repeated every 1 to 2 h, would be functionally equivalent to a continuous low dose rate irradiation, at least in terms of early responding endpoints. Longer intervals between pulses might result in loss of equivalence in some cases.

Pulsed brachytherapy, Continuous low dose rate, Human carcinoma cells.

INTRODUCTION

Both from a physical and a radiobiological standpoint, brachytherapy may well be the radiotherapeutic treatment of choice for the 5 to 10% of patients in whom the tumor is accessible for an implant. A potentially useful development in this regard has been the introduction of pulsed brachytherapy (often termed PDR—Pulsed Dose Rate), made possible by the availability of computer controlled remote afterloaders. An irradiator based on this principle consists of a single high activity radioactive source that steps through the catheters of an implant, with dwell times in each position adjusted to obtain the required dose distribution.

As currently used, a tumor is typically irradiated with 5–10-min pulses repeated at roughly hourly intervals, so that the overall dose, time, and dose rate is the same as in conventional continuous low dose rate (CLDR) brachytherapy.

This strategy results in a number of advantages, including (a) improved potential for computer-based dose optimization because of the flexibility of a stepping source and variable dwell times; (b) only a single source is used, rather than the inventory of sources that is typically required for conventional CLDR brachytherapy; (c) good radiation protection—no source preparation is involved, and the source is retracted in a shielded safe during the majority of the clock time of a treatment, allowing for routine visits; (d) the overall treatment time of implants can be standardized, as the overall dose rate can be maintained even as the source decays, by increasing the length of each pulse.

In the original article proposing the use of pulsed brachytherapy, Brenner and Hall (3) analyzed data from 36 cell lines of human origin; they suggested that a pulsed regime consisting of 0.6 Gy given in a 10-min pulse and repeated every hour would, in practice, be indistinguishable from continuous low dose rate irra-

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diation at 0.6 Gy/h, as far as early responding endpoints are concerned.

This proposal has been followed by a number of centers using pulsed brachytherapy in a clinical situation [e.g., (12, 14, 15), and, to date, no significant differences in early response have been noted.

The first published experimental test of pulsed brachytherapy was by Armour *et al.* (1), using 9L rat gliosarcoma cells cultured *in vitro*. In this cell line, no difference in cell survival could be detected between CLDR at 0.5 Gy/h and pulsed schedules up to 3 Gy every 6 h. The equivalence broke down for a pulse schedule of 6 Gy every 12 h. A comparison of CLDR and PDR was also published by Mason *et al.* (13), scoring regenerating crypts in the mouse jejunum—an *in vivo* early responding end point. They found an hourly PDR schedule to be indistinguishable from CLDR at 0.7 Gy/h, whether the pulse was delivered in 10 min or 1 min. More recent laboratory comparisons between CLDR and PDR of late sequelae (5, 6) have also not revealed significant differences.

The present investigation continues these studies and examines, *in vitro*, the equivalence of PDR and CLDR using three cell line of human origin, derived from carcinomas of the breast and cervix. The choice of cell lines reflects the current clinical use of the PDR technique. In this work, we had two related aims: 1) to test the equivalence—for an early-responding end point—between CLDR and a PDR regimen consisting of short hourly pulses; 2) to investigate the limitations of such equivalence (if any) as the period between pulses is increased beyond one hour whilst continuing to maintain a constant overall dose rate.

The rationale behind this second aim is the desire to use PDR in the clinic in the mst convenient form that still maintains equivalence with CLDR. Clearly, longer gaps between pulses are logistically more convenient.

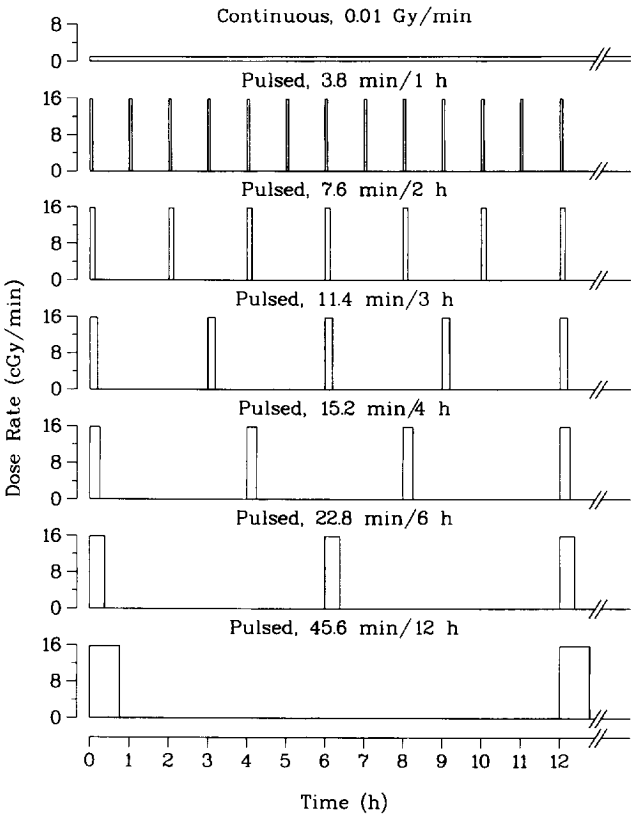

Fig. 1. Schematic of continuous low dose rate (CLDR) and pulsed dose rate (PDR) regimens used in the experiments reported here. In all cases, the overall dose rate was 0.01 Gy/min. The instantaneous dose rate of the PDR pulses was 0.158 Gy/min.

METHODS AND MATERIALS

Cell culture

There were HTB35 human cervical carcinoma cells, and MCF7 and T47D human breast carcinoma cell lines.[1] The cells were grown at in monolayers at 37°C in a humidified atmosphere of 5% CO_2–95% air. the growth medium was Eagle's minimum essential medium supplemented with 10% fetal calf serum (heat inactivated)[2] containing essential amino acids, nonessential amino acids, L-glutamine, vitamins, and gentamycin. Exponentially growing cells were seeded at densities of 50,000 to 200,000 per 35-mm Petri dish, which contained 3 ml of medium. Cells were allowed to grow for an additional 48–96 h before being irradiated and then assayed for clonogenic survival. For preliminary growth studies, cells were seeded in Petri dishes and two dishes were counted on a Coulter Counter at various subsequent times.

Irradiation

High dose rate exposures were performed with an AECL Gammacell 40 ^{137}Cs irradiator at a dose rate of 1.10 Gy/min. The CLDR irradiations were performed with a custom-made low dose rate facility (8, 9), at a dose rate of 0.01 Gy/min. PDR irradiations were carried out on the AECL irradiator with a retrofitted filter system that reduced the instantaneous dose rate to 0.158 Gy/min. A digital counter with two presets and a batch count was used to generate pulses with appropriate durations and frequencies. The cells in the irradiators were temperature controlled at 37°C, with appropriate humidity.

Each PDR irradiation was concomitantly performed with a corresponding CLDR irradiation. PDR pulse regimens were pulses of 3.8 min every hour, 7.6 min every 2 h, 11.4 min every 3 h, 15.2 min every 4 h, 22.8 min every 6 h, and 45.6 min every 12 h. In each case the total dose, total time, and the overall dose rate were the same as in the corresponding CLDR irradiation. A schematic repre-

[1] American Type Culture Collection (ATCC).

[2] SerXtend (Irving Scientific, Santa Ana, CA).

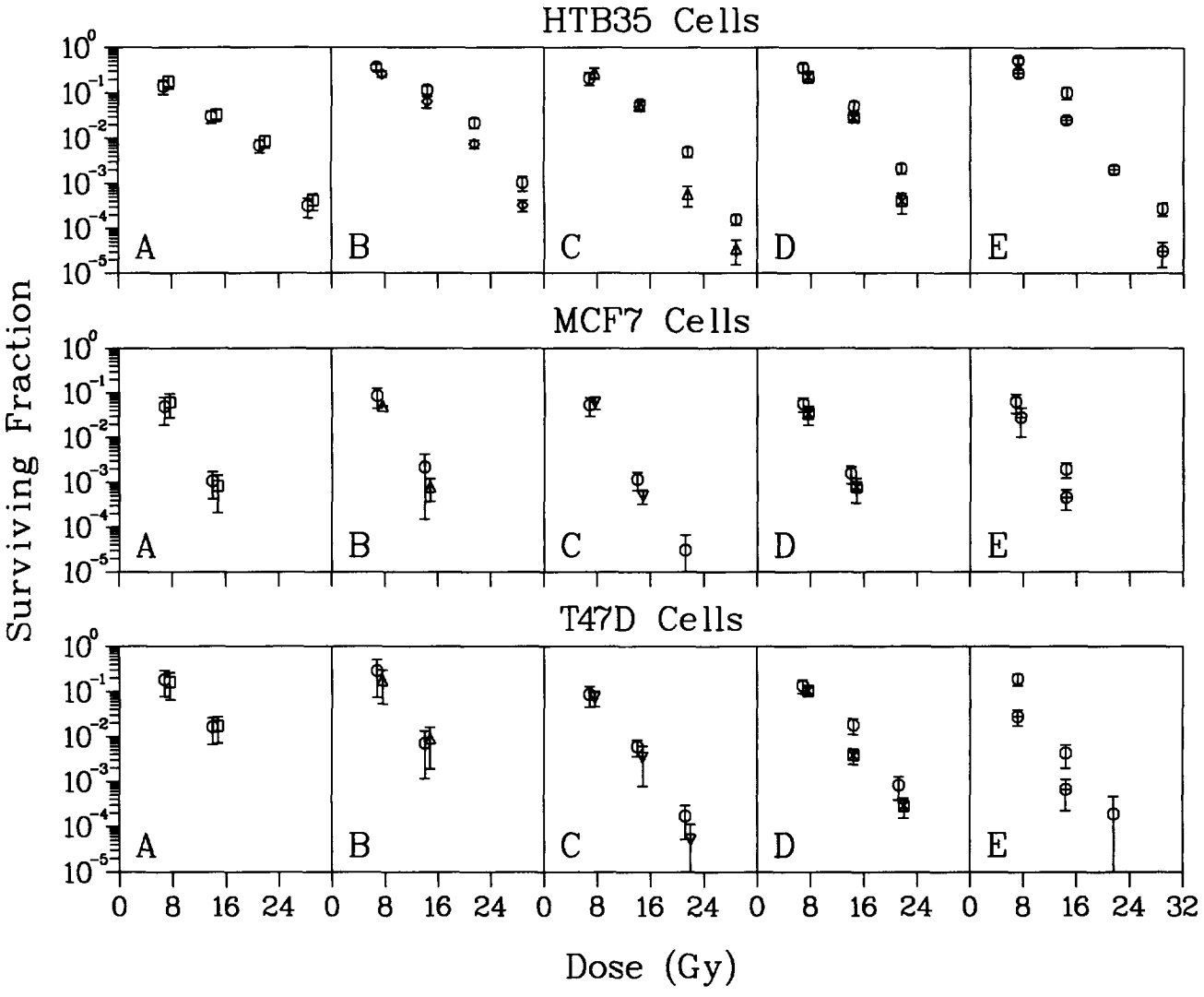

Fig. 2. Survival data for human carcinoma cells irradiated with continuous low dose rate (CLDR) and pulsed dose rate (PDR), all with an overall dose rate of 0.01 Gy/min. In all cases, open circles represent surviving fraction for CLDR, as measured in head-to-head (i.e., performed simultaneously) comparisons with the corresponding PDR regimen. For HTB35 cells (top panel), head-to-head comparisons are shown between CLDR and PDR, with pulse intervals (A) 1 h, (B) 2 h, (C) 3 h, (D) 6 h, and (E) 12 h. For MCF7 cells (middle panel), head-to-head comparisons are shown between CLDR and PDR with pulse intervals (A) 1 h, (B) 3 h, (C) 4 h, (D) 6 h, and (E) 12 h. For T47D cells (lower panel), head-to-head comparisons are shown between CLDR and PDR with pulse intervals (A) 1 h, (B) 3 h, (C) 4 h, (D) 6 h, and (E) 12 h.

sentation of the pulses is shown in Fig. 1. In all cases, comparisons were "head-to-head," i.e., simultaneous irradiations using CLDR and the appropriate PDR regimen were performed.

After exposure, the cells were trypsinized from the dishes, resuspended in medium, and counted; appropriate numbers of cells were plated into 100 20-mm Petri dishes containing a total of 10 ml medium. After 14 days of incubation, colonies were counted, and surviving fractions were estimated.

Data analysis

An important component of the experimental design and analysis is estimation of the power of the experiments. For example, a conclusion that the results from two regimens were indistinguishable could result (a) from the fact the regimens really are biologically indistinguishable (as defined in some way), or (b) from the fact that experimental was designed such that it had insufficient power to detect a significant difference, even if it were real. The details of the power calculations are given in the Appendix.

Actual statistical comparisons between the results of the CLDR exposures and the PDR exposures were done both by comparing the results of single-dose head-to-head comparisons, and by globally (i.e., for all delivered doses) comparing the results of CLDR with the corresponding PDR results with a given PDR gap between pulses.

Table 1. Results of simultaneous head-to-head comparisons between CLDR and a particular PDR regimen, for a given dose

PDR gap (h)	Dose (Gy)	p-Value (HTB35 cells)	p-Value (T47D cells)	p-Value (MCF7 cells)
1	7.2	0.88	0.39	0.78
1	14.4	0.72	0.61	0.36
1	21.6	0.88	—	—
1	28.8	0.87	—	—
2	7.2	0.00	—	—
2	14.4	0.00	—	—
2	21.6	$<10^{-2}$	—	—
2	28.8	$<10^{-2}$	—	—
3	7.2	0.91	0.04	0.04
3	14.4	0.32	0.80	0.05
3	21.6	$<10^{-2}$	—	—
3	28.8	$<10^{-2}$	—	—
4	7.2	—	0.29	0.74
4	14.4	—	0.09	$<10^{-2}$
4	21.6	—	0.05	—
6	7.2	$<10^{-2}$	0.08	0.03
6	14.4	$<10^{-2}$	$<10^{-2}$	0.02
6	21.6	$<10^{-2}$	$<10^{-2}$	—
12	7.2	$<10^{-2}$	$<10^{-2}$	0.02
12	14.4	$<10^{-2}$	$<10^{-2}$	$<10^{-2}$
12	21.6	—	0.51	—
12	28.8	$<10^{-2}$	—	—

A p-value of less than 0.05 implies rejection of the null hypothesis of biological equivalence between CLDR and that particular PDR exposure, at the 95% level of significance.

Tests to compare the single-dose paired (PDR/CLDR) results were performed using the methodology described in the Appendix. To compare globally (i.e., for all examined doses) CLDR results with those for a given PLDR gap between pulses, the likelihood ratio test was used. The null hypothesis for each test was that there was no difference in the dose–survival curve produced by a particular PDR regimen and the corresponding CLDR exposures. Under the null hypothesis, all data from each pair of CLDR/PDR regimens were fitted with a model in which the measured number of clones was assumed to have a Poisson distribution with mean

$$n\,P\exp(-\alpha D-\beta D^2), \qquad \text{(Eq. 1)}$$

where D is the dose, n the number of cells plated, P the plating efficiency, and α and β are standard linear-quadratic model parameters. Under the alternate hypothesis, the data were fitted with a model in which the measured number of clones was

$$n\,P\exp(-\alpha_j D-\beta_j D^2), \qquad \text{(Eq. 2)}$$

where the subscript j (= CLDR or PDR) refers either to CLDR or to the particular PDR regimen with which CLDR is being compared. Both models are in the form of a generalized linear model, and their maximum likelihood estimators can be calculated by the technique of iteratively reweighted least squares (10). The test statistic for the likelihood ratio test has an approximately chi-squared distribution with 2 degrees of freedom, when the null hypothesis is true. Appropriate p-values may thus be calculated.

In addition, the ratios of mean inactivation doses (MID) were used as a graphical measure of the overall difference between pairs of survival curves for continuous and pulsed irradiation (7). The MID is defined as the average dose of the differential survival probability function distribution

Table 2. p-Values for testing the equivalence of CLDR and different PDR regimens, over the entire dose range examined

	PDR pulse interval					
Cell-line	1 h	2 h	3 h	4 h	6 h	12 h
MCF7	0.71		0.034	$<10^{-4}$	0.012	$<10^{-4}$
T47D	0.91		0.15	0.011	$<10^{-4}$	$<10^{-4}$
HTB35	0.21	$<10^{-4}$	$<10^{-4}$		$<10^{-4}$	$<10^{-4}$

A p-value less than 0.05 implies that we may reject the null hypothesis of equivalence between CLDR and that particular PDR regime at the 95% level of significance.

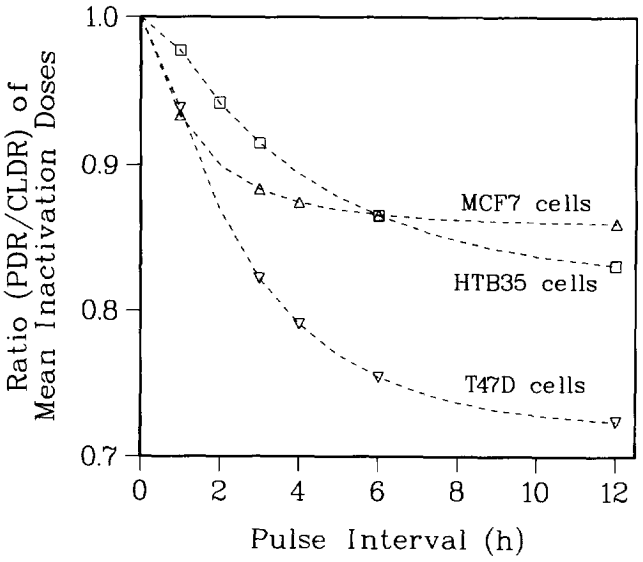

Fig. 3. Comparison of CLDR to PDR expressed in terms of the ratio of the mean inactivation doses (MID), i.e., the areas under each survival curve. The ratios are calculated based using linear-quadratic (LQ) parameters obtained from fits to all the experimental data obtained using the cell line in question. Calculations were made only for the points shown; the connecting lines are to guide the eye only. Based on Monte-Carlo simulation of the propagation of the variances of the LQ parameters, the uncertainty in these calculated MID ratios due to the uncertainties in the determination of the LQ parameters is less than 1%.

(11), and is actually the total area under the survival curve. The area was calculated using an analytic technique (2).

RESULTS

Our initial growth-curve experiments confirmed that the experimental cells, which were utilized for irradiation between days 2 and 4 postinitiation of culture, were in exponential phase. These results also showed that the cells stay in exponential growth over a 6-day period, and thus, during the time (24–48 h) of CLDR and PDR irradiations, asynchronous populations of cells will have been irradiated.

Survival results for the various regimens illustrated in Fig. 1 are shown in Fig. 2. Inspection of these figures immediately suggests that the PDR data with hourly pulses are not distinguishable from the corresponding CLDR data. However, as the time between PDR pulses increases, there is a trend towards PDR showing increasing biological effectiveness relative to CLDR. These individual comparisons are quantified in Table 1; in addition, it is important to note that the results in Table A1 (Appendix) confirm that the experimental design was such that differences in biological effectiveness, if present, would be detected.

Global comparisons (i.e., for all delivered doses) of CLDR results with corresponding PDR results for a given gap were performed as described above. The results are shown in Table 2, and again confirm biological equivalence between CLDR and PDR with 1-h gaps, but again with a trend towards loss of equivalence as the gap between PDR pulses is lengthened.

As a graphical illustration of the trends displayed in Fig. 2 and Tables 1 and 2, in Fig. 3 the results are expressed in terms of the ratio of the mean inactivation doses (MID) for the various PDR regimens and CLDR.

DISCUSSION

From an analysis of dose rate data on many cell lines of human origin, Brenner and Hall (3) suggested that PDR with a 10-min pulse every hour would be indistinguishable from CLDR at 0.6 Gy/h. This conclusion was based on calculations for each cell line using the values of radiobiological parameters obtained from analyzing dose rate data. Of four subsequent experimental checks of PDR in the literature, two corroborated the equivalence of CLDR and PDR for hourly pulses, but did not investigate longer pulse intervals (6, 13), while two have suggested that longer pulse intervals could be used while still maintaining biological equivalence (1, 5).

In the present study, it is clear that there is no significant difference in cell survival between PDR and CLDR, for any of the cell lines, when a pulse interval of 1 h is used. As the pulse interval increases, PDR becomes more effective than CLDR, for a given dose, and there is a significant differences for pulse intervals of 6 and 12 h.

While times between pulses that are considerably longer than 1 h would be more convenient in a clinical setting, and may be acceptable in many cases, the variability between cell lines supports the initial conservative suggestion (3) that hourly, or probably two hourly, pulses would be expected to result in effective equivalence between PDR and CLDR, at least for early effects, for a wide spectrum of cell lines of human origin. Longer pulse intervals may well involve potentially significantly increased biological effectiveness in some small subset of cases.

Finally, it is stressed that the *in vitro* studies described here represent model systems for early responding effects in the clinic, including tumor control. Late effects are, of course, of major concern and must also be assessed carefully (3–6).

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APPENDIX

In the section, we will describe a statistical procedure of testing whether the surviving fractions after CLDR and PDR are equal, and we also calculate the probability that our experimental design would be able to detect biological differences, should they exist, between the results of head-to-head CLDR/PDR experimental comparisons.

We first introduce some notations. Let n denote the number of cells plated, Y the number of colonies observed,

Table A1. Power of the experimental design; tabulated is the probability of rejecting the null hypothesis (of equal biological effectiveness), when survival after a given PDR exposure is significantly different (based on Equation A1) from that measured for the corresponding CLDR exposure

PDR gap (h)	Dose (Gy)	Power of test (HTB35 cells)	Power of test (T47D cells)	Power of test (MCF7 cells)
1	7.2	0.939	0.773	0.589
1	14.4	0.963	0.747	0.723
1	21.6	0.988	—	—
1	28.8	0.993	—	—
2	7.2	1.000	—	—
2	14.4	0.989	—	—
2	21.6	0.987	—	—
2	28.8	0.997	—	—
3	7.2	0.951	0.826	0.760
3	14.4	0.997	0.738	0.546
3	21.6	0.990	—	—
3	28.8	1.000	—	—
4	7.2	—	0.878	0.886
4	14.4	—	0.674	0.989
4	21.6	—	0.716	—
6	7.2	1.000	0.991	0.865
6	14.4	1.000	0.786	0.937
6	21.6	1.000	0.952	—
12	7.2	1.000	0.957	0.611
12	14.4	0.935	0.678	0.956
12	21.6	—	0.399	—
12	28.8	0.999	—	—

The calculations are for tests performed at the 95% significance level.

P the plating efficiency, and S the surviving fraction (we attach subscripts C and P to n, Y, and S, to denote head-to-head treatments with CLDR and PDR, respectively). The plating efficiency, P, is common in both treatments for head-to-head comparisons.

Following (3), we consider S_C and S_P to be insignificantly different when

$$S_P > S_C/(-\log S_C), \text{ if } S_C < 0.01,$$

or

$$S_P > 0.5\ S_C, \text{ if } S_C \geq 0.01, \qquad \text{(Eq. A1)}$$

noting that, in general, we expect $S_P \leq S_C$.

Assume that the number of cells plated is a Poisson random variable with mean n, then the number of colonies observed is a Poisson random variable with mean nPS. We wish to test the null hypothesis H_0: $S_C/S_P = 1$ vs. the alternate hypothesis H_1: $S_C/S_P > 1$. That is, we are to decide, on the basis of the outcomes Y_C and Y_P of a experiment, whether to accept H_0 (reject H_1), or to reject H_0 (accept H_1).

It can be shown that the conditional distribution of Y_C, given the total number of colonies ($Y_C + Y_P = t$) measured in a head-to-head comparison, is the binomial distribution corresponding to t trials with probability of success $\theta = n_C S_C/(n_C S_C + n_P S_P)$. Conditional on $Y_C + Y_P = t$, the test we are interested in becomes one of testing the parameter θ of a binomial distribution. The two hypotheses become respectively H_0: $\theta = \theta_0$ and H_1: $\theta > \theta_0$, where $\theta_0 = n_C/(n_C + n_P)$.

A statistical test may commit two types of errors: type I errors of rejecting H_0 when it is true, and type II error of accepting H_0 when it is false. We will limit our attention to tests such that the probability of type I errors is not greater than α, where α is an appropriate level of significance.

Clearly, we should reject H_0 when the observed Y_C is too large, under the assumption that the null hypothesis H_0 is true. To be specific, we use a test that accepts H_0 if $Y_C < c$, and rejects H_0 if $Y_C \geq c$. The quantity c is called the critical value. We define the power function $\pi(c,r,t)$ for such a conditional test with critical value c as the probability of rejecting the null hypothesis H_0 when $S_C/S_P = r$. It is easy to show that

$$\pi(c,r,t) = \sum_{j=c}^{t} \binom{t}{j} \theta^j (1-\theta)^{t-j} \qquad \text{(Eq. A2)}$$

where $\theta = n_C r/(n_C r + n_P)$.

Because Y_C has a discrete binomial distribution conditioned on $Y_C + Y_P = t$, we cannot, in general, find a critical value c such that the size of the test equals exactly to α unless a randomized decision is allowed; in this analysis, we first find k such that $\pi(k,1,t) \geq \alpha$ and $\pi(k+1,1,t) < \alpha$, then let $c = k$ if $\pi(k,1,t)$ is closer to α, or let $c = k+1$ if $\pi(k+1,1,t)$ is closer to α.

Thus, to calculate the power of any given experiment, we choose a significance level $\alpha = 0.05$, and use n_C, n_P, P, and S_C as derived from the experiment, and $r = -\log S_C$ if $S_C < 0.01$, or $r = 2$ otherwise.