replexa+ *Consistent, Positive Outcomes*

Replexa+ Cellular Data Report: In Vitro Study

Protocol Purpose and Justification

The purpose of the test was to determine the optimal Shortwave Diathermy configuration and Applicator size. Optimization was determined by the effect of increasing cell growth of normal human fibroblasts in vitro.

- Shortwave Diathermy configuration can consist of many differnt Power, Pulse Width and Pulse Duration Combinations
- Applicator size tested: small and large

Test Set Up

This test was performed by an independent science lab in Nashville, TN.

Adult Human Dermal Fibroblast (HDF) Cell Cultures were used. Routine culture was performed as recommended by the manufacturer in a 5% CO_2 humidified incubator at 37°C. Cells were cultured in minimum essential media (MEM) supplemented with 100 units penicilin, 100 µg streptomycin, and 5% FCS.

Cells were used for experimentation after 16 hours in a humidified incubator at 37°C with 5% CO_2. Cells were used for experimentation from passage 2-6 (3-8 population doublings). The cells were plated 16 hours prior to treatment.

Test Protocol - Cell Proliferation Assay

1. Make up a concentrated suspension in growth media.
2. Serial dilute the cell suspension so that in 100 µl either 1 X 103, or 5 X 103, or 1 X 104 cells will be added to 20 independent wells for each cell density.
3. Allow the cells to plate for 12-14 hours.
4. Treat the cells at 5.08 cm above the treatment applicator separated by a saline solution (0.18%NaCl).
5. Treat the cells for one 30-minute cycle.
6. All the cells to grow after treatment for 12-16 hours.
7. Wash the cells 2X with PBS and freeze at -80°C for at least 1 hour.
8. Add 100 µl of the CyQuant lysis/assay reagent and assay at 480 nm excitation/520 nm absoprtion excitation.

Quantization was performed using a Victor fluorescent spectrophotometer (Perkim Elmer) using an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Counts were generally >30,000 U above background. The data was normalized to control samples (untreated).

Statistics

Analysis of variance (ANOVA) or Student's T-test was used to test for overall statistical significance. Comparison between groups with an n>100 was made. Both methods gave similar results.

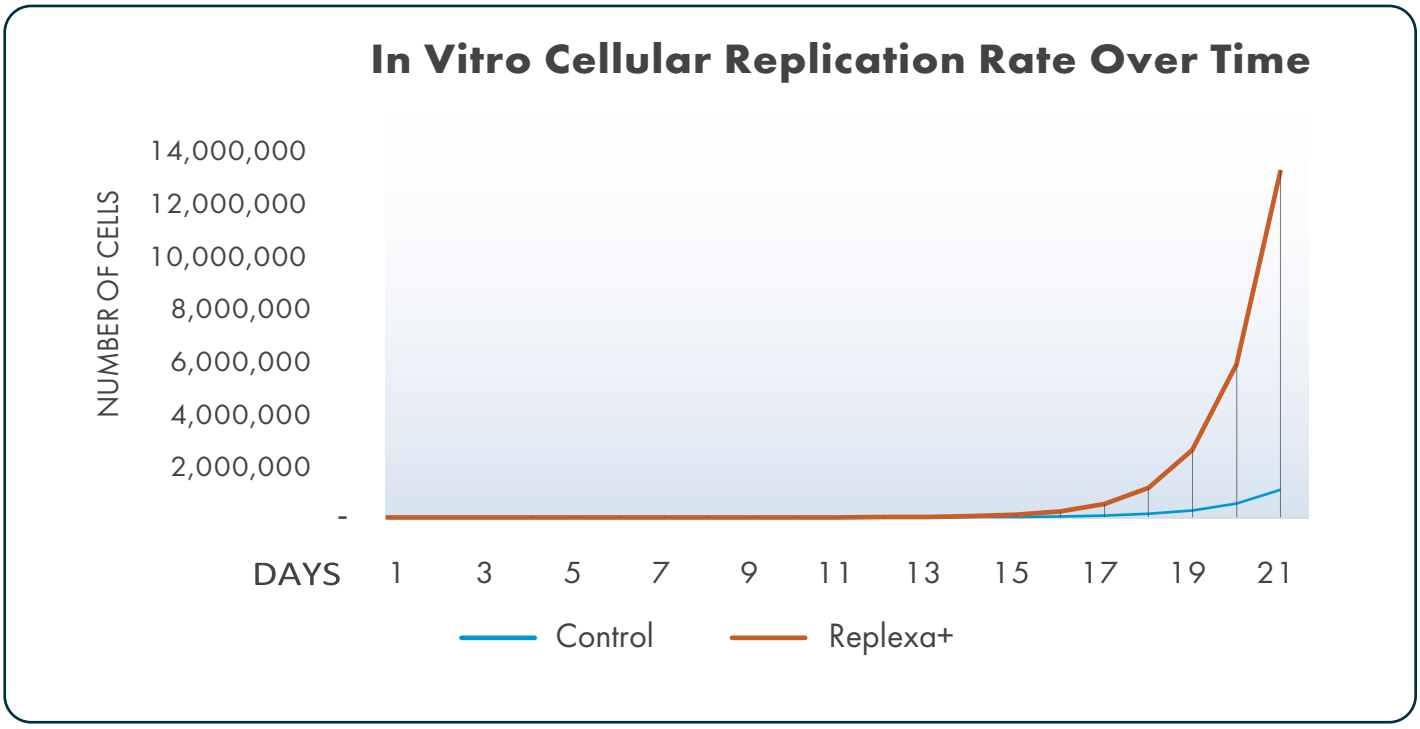
Testing for Shortwave Diathermy Configuration and Applicator Size

Eighteen (18) different combinations of Shortwave Diathermy configuration and Applicator size were tested. Each combination was treated in five separate tests. Each test included one Test Subject Plate compared to five Control Plates. Each Plate contained 20 light density wells (2.5 X 103 cells/well) and 20 heavy density wells (5.0 X 103 cells/wells).

» Total tests conducted = 21, 600

Conclusion - Replexa+ Provides Exponential Results

Replexa+ can increase cell growth of normal human fibroblasts by 27% over non-treated controls ($p<0.001$). The ability to stimulate and enhance cellular performance requires time to be effective but it then provides an exponential, positive effect to the treatment site.



Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	1	2	4	8	16	32	64	128	256	512	1,024	2,048	4,096	8,192	16,384	32,768	65,536	131,072	262,144	524,288	1,048,576
Replexa+	1	2	5	12	27	60	137	311	705	1,600	3,633	8,247	18,720	42,495	96,463	218,972	497,066	1,128,339	2,561,329	5,814,218	13,198,274

*Growth rate calculated using the most conservative figures from testing. Testing data available upon request.

PROMEDTEK

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866-388-2410
480-385-2499

4110 N Scottsdale Rd
Suite 270
Scottsdale, AZ 85251