BTX Applications

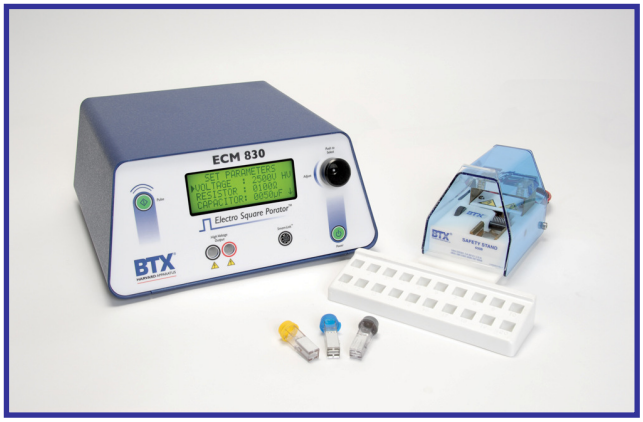
Tech-Trends

Volume 3, Series 10

Electro-molecular targeted stem cell therapy

Electro-Molecular Therapy using Adult Mesenchymal Stem Cells

Abstract— Clinically chemo-refractive types of cancers do not respond well to conventional therapies. To treat and enhance the efficacy of drug delivery for these cancers, we have developed an *in vitro* model of a combination therapy using adult Mesenchymal stem cells. Adult Mesenchymal stem cells have been used for this study primarily because of their ability to home towards tumor cells, making the possibility to practice targeted tumor therapy more realistic. These cells, derived from Human adult bone marrow were subjected to high intensity, short duration (1200V/cm, 100µs), and low intensity, long duration (200V/cm, 40ms and 450V/cm, 25ms) pulses. The effect of these voltages on the viability and proliferation ability of these cells in the presence and absence of Bleomycin (FDA approved chemodrug used for treating various cancers) indicate the possibility of transfer of this technique to clinical practice for effective electro-molecular targeted stem cell therapy. An analysis of the electrical energy applied vs. the viability illustrates a linear relationship. The dose curve exhibits a non-linear relationship. These results indicate that the high efficacy of MSC targeted combination therapy would provide efficient, economical, and enhanced clinical benefit for many types of cancers which need alternate treatments.



450002 ECM 830 Square Wave System

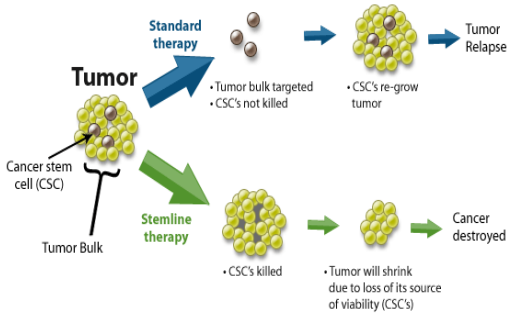


Figure 1: Cancer stem cells (CSCs) are cancer cells (found within tumors or hematological cancers) that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs are therefore tumorigenic (tumor-forming), perhaps in contrast to other non-tumorigenic cancer cells

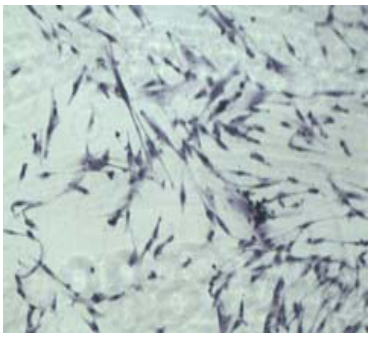


Figure 3: Adult Mesenchymal Stem cells from a 42 year old male patient .

Cell Preparation: Human bone marrow aspirates from an adult patient (54 year old male) undergoing cardiac surgery were collected after preinformed consent from the patient and due approval from the Institutional Ethics Committee. They were collected from Sternal bone using an aspirating needle. Fig. 3 shows the morphology of an adult hMSCs [10]. The bone marrow sample was carefully overlaid onto the Ficoll Hypaque column. The sample was centrifuged at 1800rpm for 20 minutes after a 1:1 dilution with DMEM. The upper layer was aspirated leaving behind the mononuclear cell layer at the interphase. The Buffy coat was transferred to another tube and the volume was made up to 10ml with PBS. The contents were centrifuged at 1800rpm for 10 minutes. After 2-3 washes with PBS, the supernatant was discarded & the pellet was then used for expansion. The cells were seeded at approximately 1 x 10^6cells/mL density in 90 mm cell culture dishes containing low glucose DMEM, 10% FBS, L-Glutamine and antibiotics. The dishes were maintained at 37ºC in a humidified atmosphere of 95% air and 5% CO_2. The cells are centrifuged again at 2000rpm for 5 minutes and resuspended to required volume for experimentation.

Electroporation Settings:

Choose Mode:	LV
Set Voltage:	480 V
Set Pulse Length:	100 us
Set # of Pulses:	8
Electrode gap:	4 mm
Field Strength:	1200 V/cm

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Human mesenchymal stem cells and bleomycin in vitro electroporation

A. Dose Curve

Most of the chemodrugs have a lifetime quantity that should not be exceeded. For example, not more than 400 units of bleomycin should be administered to a patient in the entire life time. Hence, a dose curve study was performed to identify the efficacy of various doses of the drug and their viabilities for all the three pulse parameters. Fig. 6 shows the dose curve for 200V/cm, 40ms pulse parameters. The viability of cell population decreases with the increase of the dose of the chemodrug. For an order of magnitude change in the dose, the viability varies about 20%, illustrating the non-linear relationship. Fig. 7 shows a comparison of the dose curves of the pulse parameters studied.

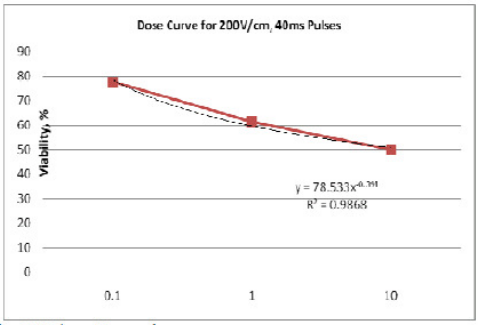

Fig. 6. Dose curve for 200V/cm, 40ms pulse parameters.

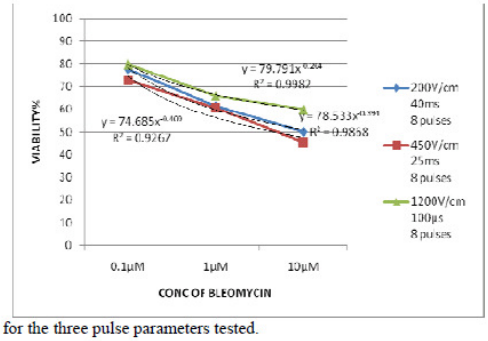

Fig. 7. Dose curve for the three pulse parameters tested.

Electro-Molecular Therapy using Adult Mesenchymal Stem Cells

Kavitha Sankaranarayanan[1], Raja Prabhu Ramachandran[2], M Sriram Kumar[2], V Madan Kumar[2], S Vignesh[2], V Malini[2], J Chris Maria Renny[1], Soma Guhathakurta[1], K.M.Cherian[1], Arutselvan Natarajan[3], and Raji Sundararajan[4]

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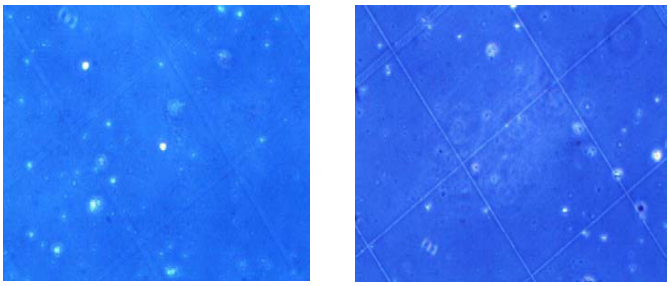
[1]Frontier Lifeline, Chennai, India
[2]B.S. Abdur Rahman University, Chennai, India
[3]Stanford Medical School, CA, USA
[4]Purdue University, West Lafayette, IN, USA

B. Viability Study

Viability is computed as the ratio of the number of live cells to the total number of cells (live and dead). The percentage viability of the cells under all the experimental conditions are given in Table I. It can be seen that 450V/cm, 25 ms, 8 pulses was the most intense pulse for all three concentrations of the drug followed by 200V/cm, 40ms, 8 pulses. The 1200V/cm, 100µs, 8 pulses showed the least mortality. This phenomenon can be explained by computing the amount of energy delivered for each pulse set as given below. Fig. 8 shows the live and dead cells using trypan blue exclusion assay. There are more live cells in the control than in the pulsed cell population at 200V/cm, 40ms, 8 pulses. Similar results were obtained for other pulse parameters (data not shown).



Control 200V/cm, 40ms, 8 pulses

Figure 8 Trypan blue exclusion assay showing the dead (dark spots) and live (bright spots) cells.

TABLE 1: VIABILITY VARIATION FOR VARIOUS STUDY CONDITIONS

Electric field intensity, V/cm	Pulse width, T	Bleomycin Dose	Viability
Control (none)	-	-	93.7%
Bleomycin only	-	1µM	87.0%
EP only 200V/cm	40ms	-	83.3%
EP only 450V/cm	25ms	-	81.8%
EP only 1200V/cm	100µs	-	84.6%
200	40ms	0.1µM	77.7%
450	25ms	1.0µM	72.7%
1200	100µs	10.0µM	80.0%
200	40ms	0.1µM	61.5%
450	25ms	1.0µM	60.5%
1200	100µs	10.0µM	66.0%
200	40ms	0.1µM	50.0%
450	25ms	1.0µM	45.5%
1200	100µs	10.0µM	60.0%