



PROJECT REQUIREMENT:

- To measure the wound healing capacity on L929 cell lines, 1 test compound was received.
- The sample details as follows:

Sl. No.	Sample Name	Sample code	Cell line
1	GAIN-IT	GAIN-IT	L929
2	Cipladine	Std control-50ug/ml	L929

Table 1: Details of samples received for the study

BACKGROUND OF THE STUDY:

The scratch wound healing assay has been widely adapted and modified to study the effects of a variety of experimental conditions, for instance, gene knockdown or chemical exposure, on mammalian cell migration and proliferation. In a typical scratch wound healing assay, a “wound gap” in a cell monolayer is created by scratching, and the “healing” of this gap by cell migration and growth towards the centre of the gap is monitored and often quantitated. Factors that alter the motility and/or growth of the cells can lead to increased or decreased rate of “healing” of the gap (Lampugnani, 1999). This assay is simple, inexpensive, and experimental conditions can be easily adjusted for different purposes. The assay can also be used for a high-throughput screen platform if an automated system is used (Yarrow and Perlman, 2004).

MATERIALS:

- Cell lines: L929 – Mouse embryo fibroblast cell line (NCCS, Pune)
- Cell culture medium: DMEM high glucose - (#AL111, Himedia)
- Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
- Fetal Bovine Serum (#RM10432, Himedia)
- D-PBS (#TL1006, Himedia)
- Test compound: 1 (provided by client)
- Cipladine (Commercially available)
- 12 well cell culture plate (Biolite - Thermo)
- 50 ml centrifuge tubes (# 546043 TORSON)
- 1.5 ml centrifuge tubes (TORSON)
- 10 ml serological pipettes (TORSON)
- 10 to 1000ul tips (TORSON)
- 70% ethanol

EQUIPMENTS:

1. Centrifuge (Remi: R-8°C).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
3. Inverted Biological Binocular Microscope (Biolinkz)



4. 37°C incubator with humidified atmosphere of 5% CO₂ (Healforce, China)
5. Biosafety Laminar Hood (Healforce, China)

SOFTWARE:

1. Image J
2. Windows Paint

STEPS FOLLOWED:

1. Grow cells in DMEM with high glucose media supplemented with 10% FBS until the cells reach 70-80% confluence.
2. Seed cells into 12 well tissue culture plate at a density of 0.25 million cells per well, until they reach ~80-100% confluence as a monolayer for the incubation period of 24hrs.
3. Do not change the medium. Gently and slowly scratch the monolayer with a new 200ul pipette tip across the centre of the well. While scratching across the surface of the well, the long-axial of the tip should always be perpendicular to the bottom of the well.
4. The resulting gap distance therefore equals to the outer diameter of the end of tip. The gap distance can be adjusted by using different types of tips. Scratch a straight line in one direction.
5. Scratch another straight line perpendicular to the first line to create a cross in each well.
6. After scratching, gently wash the well twice with medium to remove the detached cells.
7. Treat the cells with desired concentrations of given compound prepared in media and incubate at 37°C with 5% CO₂ in the incubator.
8. Grow cells for additional 48hours (or the time required if different cells are used).
9. Capture the cell images at different time intervals (ex: 0,12hr, 24hr., etc)
10. Set the same configurations of the microscope when taking pictures for different views of the monolayer. The gap distance can be quantitatively evaluated using software such as Image J. To reduce variability in results, it's suggested that multiple views of each well should be documented, and each experimental group should be repeated multiple times.

FORMULA USED FOR THE ANALYSIS:

% Wound Healing Score = (Initial area-Final area)/Initial area *100



RESULTS:

In this study, given 1 test compound was evaluated to analyse the wound healing efficacy on L929 cells. The concentrations of the test compound used to treat the cells are as follows:

Sl.No	Culture condition	Cell lines	Concentration treated to cells
1	Untreated	L929	No treatment
2	Blank	-	Only Media without cells
3	Cipladine	L929	50ug/ml
4	Test compound	L929	100ug/ml

Table 2: Details of drug treatment to respective cell lines used for the study

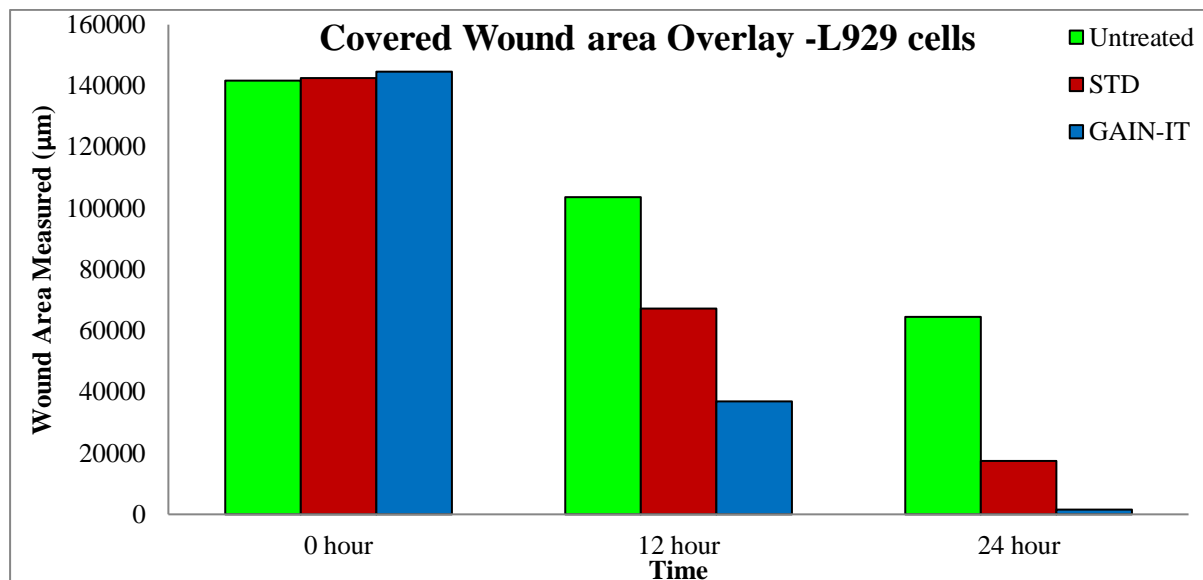
OBSERVATIONS & CONCLUSIONS:

Wound Area Covered Overlay-L929 cells			
Incubation	Untreated	STD	GAIN-IT
0 hour	141760.889	142537.053	144709.5066
12 hour	103674.2836	67225.3504	36811.0084
24 hour	64570.13	17415.983	1451.3318

Table-3: Wound healing area values of GAIN-IT treated L929 cells after the treatment period of 12hrs and 24hrs.

- The results of Wound healing assay suggest that the given test compound, GAIN-IT was effective healing potency in nature on Mouse embryo fibroblasts (L929) which decreased the area of wound on incubation dependent manner respectively.
- The observed wound area with calculations are enclosed in MS excel format.
- The direct microscopic observations of images of test compound, standard control after 0hour, 12hour and 24 hour of incubation were enclosed in the separate folder along with this report.

OVERLAY GRAPH:



Graph 1: Measured wound area values of GAIN-IT, Cipladine treated cells in comparison to the untreated cells after the different incubation periods of 0 hours, 12hours and 24hrs.

Overlay % Wound Closure scored - L929 cells			
Incubation	Untreated	STD	GAIN-IT
0 hour	0	0	0
12 hour	26.86679356	52.83657912	74.56213537
24 hour	54.45137904	87.78143466	98.99707225

Table-4: % Wound closure values of GAIN-IT treated L929 cells after the treatment period of 12hrs and 24hrs.

CONCLUSION:

The in vitro Wound healing assay results suggest us that the given test compound, was non-toxic as well as cell proliferative in nature on Mouse embryo fibroblast cells. After 12hours of incubation, GAIN-IT and Cipladine showed wound healing potency of 74.56% and 52.83% after 12hours and 87.78% and 98.99% after the 24hours respectively.

GAIN-IT molecule exhibited effective wound healing properties better than the commercially available wound healing drug, Cipladine and further studies have to be conducted to determine the molecular mechanism behind Wound healing efficacy properties of the test compound at in vitro level by measuring the level of Collagenase and Elastase by Spectrophotometry method.



Enclosures:

- 1) **Excel Sheet with Calculations and Raw Data**
- 2) **Captured Cell Images at different intervals with the measured area**