國泰綜合醫院
Cathay General Hospital

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

Test Report

Cytotoxicity Test of A New JM Nanocomposite Material on Neonatal Human Dermal Fibroblasts

Test Reagent
New JM nanocomposite material

Project Commissioner
JM Material Technology Inc.

Project Implementation Unit
Cell Biology Laboratory, Cathay Medical Research Institute, Department of Medical Research, Cathay General Hospital

Testing Laboratory
Cell Biology Laboratory, Cathay Medical Research Institute, Department of Medical Research, Cathay General Hospital

Project Personnel
Mu-hua Chung, Qing-dong Ling

Principal Investigator
Qing-dong Ling

Signature: 

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

Abstract

Title: Cytotoxicity Test of A New JM Nanocomposite Material

Experiment design: This study tested a new JM nanocomposite materials (JMs) of various concentrations for cytotoxicity and short- and long-term effects. In the experiment, the number of living cells after direct contact with various concentrations of the JM was counted, and related biochemical reactions were observed. Subsequently, MTT crystal staining was performed to test the effect of enzyme activity on a substrate to determine cell viability under short-term (24 h) and long-term (5 d) use of the JM in order to explore the cytotoxicity of the JM on neonatal fibroblasts along with its effect on cell growth.

Testing reagent: New JM nanocomposite material

Reagent vendor: JM Material Technology Inc.,5F.-3, No. 40-2, Sec. 1, Minsheng N. Rd., Guishan Township, Taoyuan County

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

Test Content

Experiment Materials and equipment

Cell line source

Neonatal human foreskin fibroblasts (NuFF1) sourced from GlobalStem (product code: GCS-3002)

Cell observation and cell counting instruments:

Millipore Scepter cell counter

High-performance inverted microscope

MTT analysis method:

Spectrophotometry

Experimental method

1. Cell culture

   Cultivate the cell line in a 96-well culture plate (20,000 cells/well) and 24-well culture plate (30,000 cells/well). Incubate cells in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) at 36 °C with 5% CO_2 for 12 h until the cells are attached. Discard the culture fluid, rinse twice with phosphate buffer saline (PBS), and set aside.

2. JM material culture fluid

   Control group

   Add 10% ddH2O with 90% DMEM to the culture fluid (no JM added) to prepare the 0% JM culture fluid.

   Experimental group

   (a) Add 100 μL of the JM to 100 μL of the ddH_2O at an equal ratio and dilute at a ratio of 1:2.

   (b) Add 100 μL of diluted JM fluid to 900 μL of DMEM to obtain a 10% dilution, and perform a 2-fold serial dilution to prepare seven concentrations of JM culture fluid (10%, 5%, 2.5%, 1.25%, 0.625%, 0.3125%, and 0.156%).

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

(c) Separate the JM culture fluid into two groups; place one group under UV irradiation at room temperature and cover the other with aluminum foil to keep it away from light.

	1	2	3	4	5	6	7	8	9
None UV (%)	10	5	2.5	1.25	0.625	0.3125	0.156	0	Blank
UV (%)	10	5	2.5	1.25	0.625	0.3125	0.156	0	Blank

Blank group

Add only the 0% JM culture fluid to the well without any cells as a control.

3. Experiment and analysis

Short-term test

Add the various concentrations of the JM culture fluid to the experimentation cells (200 μL/well) in the 96-well culture plate; test each concentration in triplicate. Incubate at 36 °C with 5% CO_2 for 24 h. Discard the culture fluid, rinse twice using PBS, and then perform the MTT test.

Long-term test

Add the JM culture fluid (0.3125% with UV, 0.3125% without UV, 0%) to the experimentation cells (500 μL/well) in the 24-well culture plate; test each concentration tested in triplicate. Incubate at 36 °C with 5% CO_2 for 5 d. Discard the culture fluid, rinse twice using PBS, and then perform the MTT test.

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

MTT test

(a) Add the MTT solution to each culture well of the cell culture plate (50 μL/well for the 96-well plate; 200μL/well for 24-well plate).

(b) Incubate at 37 °C in the cell incubator for 1 h to test the effect of cell enzyme activity on the substrate.

(c) Cultivate for 1 h and remove the fluid from the cell culture dish.

(d) Add DMSO (100 μL/well for the 96-well plate; 400 μL/well for the 24-well plate), and place the cell culture plate in a homogenizer and shake slowly for 15 min to mix the liquid homogenously, dissolving the violet crystals in the liquid.

(e) Under an OD595 wavelength, use a spectrophotometer to measure the absorbance. Record and compare the measurements.

Interpretation and calculation

(a) Use a BioTek microplate spectrophotometer to scan the culture plate.

(b) Use Gen5 Version 1.09 to measure and record the absorbance.

(c) Substitute the experimental values in the following equation using the absorbance value of the control group as the baseline data to obtain the cell viability:

$$[\frac{\text{experimental group absorbance}}{\text{control group absorbance}} \times \% =$$

corresponding cell viability(%)]. Subsequently, use this value to calculate and construct a chart.

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

Cell staining test

(a) Cultivate the cell lines in 3.5-cm cell culture plates (700 000 cells/well), add DMEM with 10% FBS and 1% P/S, and incubate at 36 °C with 5% CO_2 for 12 h to facilitate cell attachment.

(b) Discard the culture fluid, rinse twice with PBS and add the 5%, 2.5%, 0.625%, and 0% JM culture fluids. Incubate at 36 °C with 5% CO_2 for 24 h.

(c) Discard culture fluid, rinse twice with PBS and add 4% PFA for cell fixation at 40 °C, fix cells for 1 h.

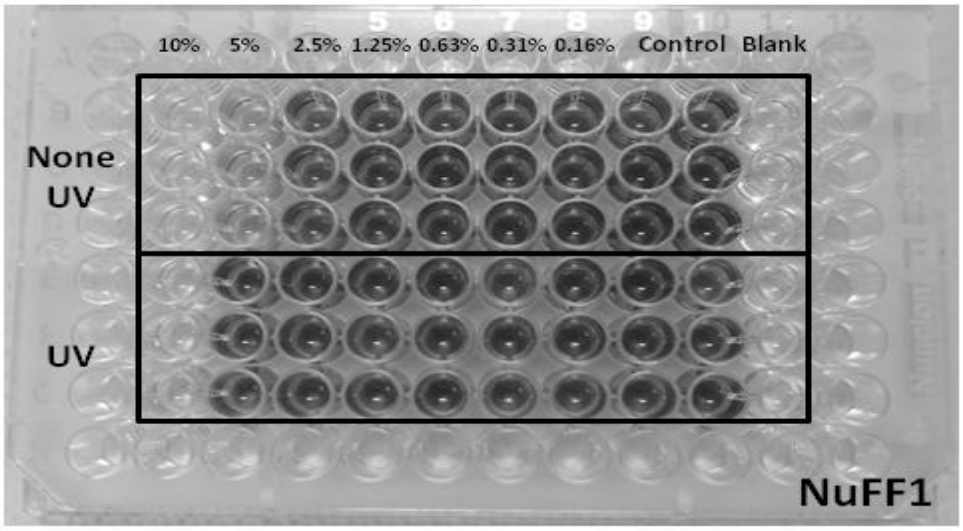
(d) Discard the 4% PFA cell fixation fluid, rinse twice with PBS, then add trypan blue stain and place on a ceramic heating plate at 50 °C, staining for 10 min.

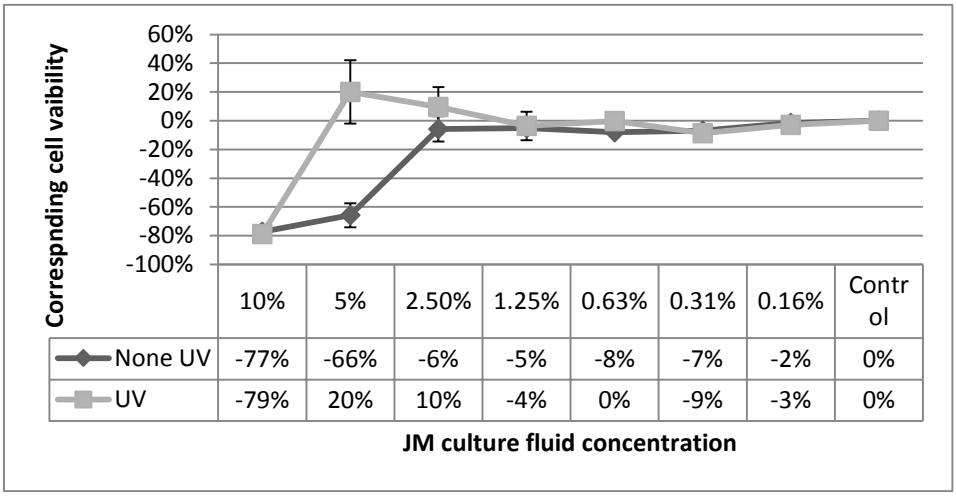
(e) Discard the trypan blue dye, rinse twice with PBS, and use a high-performance inverted microscope to observe and record the cell morphology.

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

Test Results

Short-term test



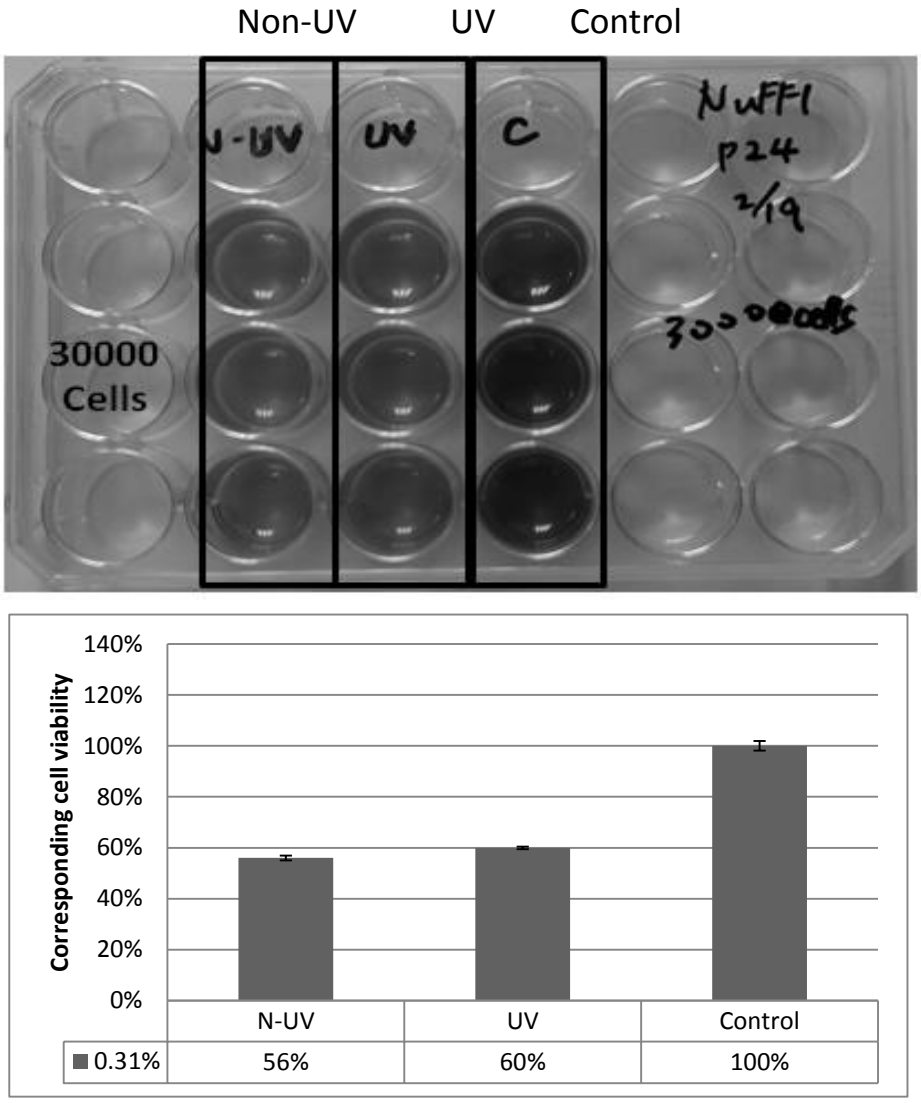


	10%	5%	2.50%	1.25%	0.63%	0.31%	0.16%	Control
None UV	-77%	-66%	-6%	-5%	-8%	-7%	-2%	0%
UV	-79%	20%	10%	-4%	0%	-9%	-3%	0%

In this experiment, the 10% JM with UV and without UV irradiation showed significantly reduced cell viability (79% and 77%, respectively) and high cytotoxicity compared with the control group. For the 5% JM, the cell viability in the JM without UV irradiation was reduced significantly (up to 66%). In the JM with UV, cell viability was only slightly reduced (9%); thus, cytotoxicity for the latter was significantly reduced. When the concentration was reduced to ≤2.5%, the cell viability of the JM with and without UV was similar to that of the control group, displaying no apparent cytotoxicity.

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

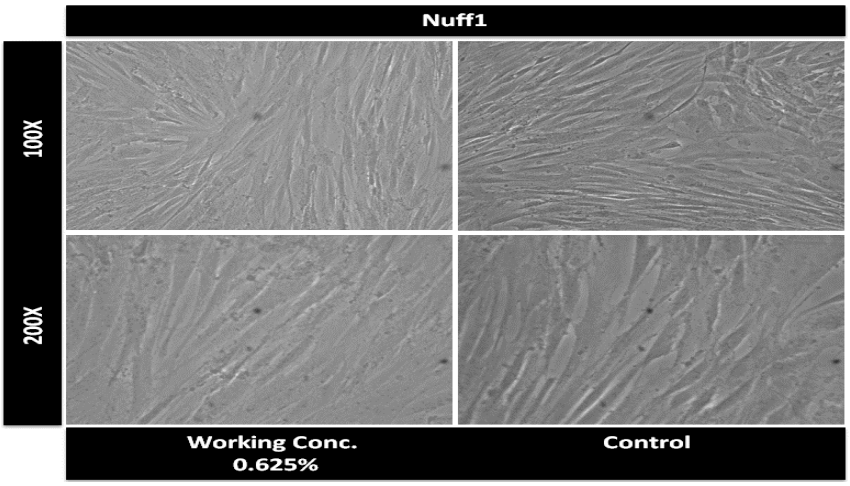
Long-term test



In the long-term experiment, cells cultivated in the 0.625% JM culture fluid with and without UV exposure retained 60% and 56% cell viability after 5 d, although decreases in cell volume were observed. Thus, the JM nanomaterials have an inhibitory effect on cell growth and proliferation.

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

Cell morphology comparison



1. Cell morphology comparison of the JM-treated working concentration (0.635%) and control group (0%) at varying magnification levels.
2. Nuff1 cell morphology remained intact and showed no difference under the various concentrations.

國泰綜合醫院
10633台北市仁愛路四段280號
Cathay General Hospital
No.280, Sec.4, Ren Ai Road,
Taipei 10633, Taiwan, R.O.C.
Tel : 02-27082121
www.cgh.org.tw

Conclusion

1. For the JM tested on the neonatal human dermal fibroblasts for 24 h, the 10% JM culture fluid with UV irradiation as well as the ≥5% JM culture fluids without UV irradiation had a cytotoxic effect.
2. Cells cultivated in the ≤2.5% JM culture fluids both with and without UV irradiation **did not show cytotoxicity**.
3. In the long-term cell culture test, cells cultivated for 5 d in the 0.625% JM culture fluid with or without UV irradiation (estimated concentration) displayed a decrease in cell volume (60% and 56%, respectively). **Thus, the JM nanomaterial inhibit cell growth and proliferation of neonatal human dermal fibroblasts.**