Supplementary Information

Gelatin-Methacryloyl Hydrogel based blood brain barrier model for studying breast-brain metastasis

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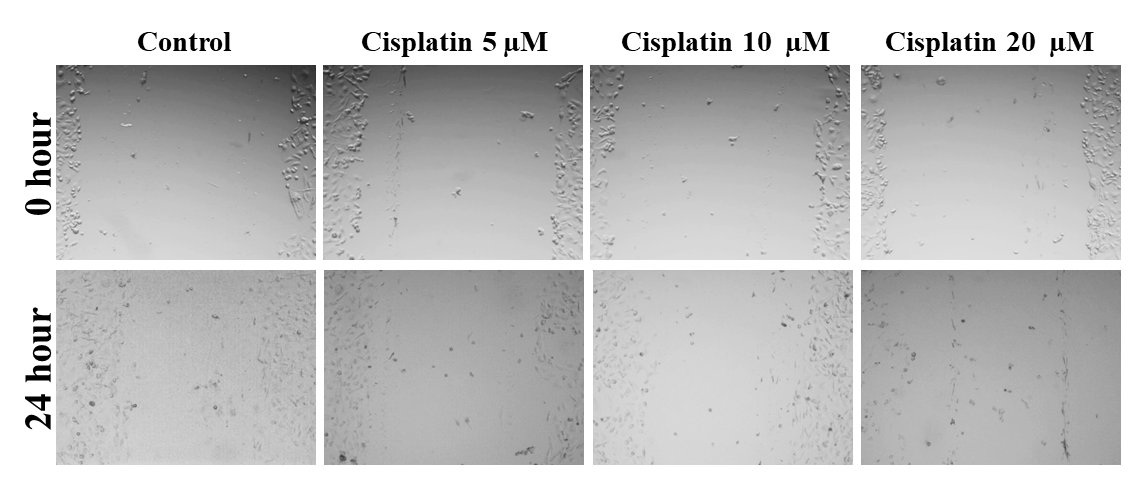
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***Migration assay***

**Experiment:** This assay was performed using GFP-MDA-MB-231 cells to determine the effect of cisplatin on cell migration. Cells were cultured in 12-well cell culture plates in serum containing DMEM medium with 1% penicillin-streptomycin-neomycin solution. Then, a scratch was made in the middle of every well with the help of pipet tip (100-µL). After washing and media replacement, cisplatin at 5 µM, 10 µM and 20 µM concentrations were applied in each well. Scratched areas were imaged using a microscope (Leica DMi1 Inverted Microscope) immediately after the procedure and after 24 hours.

**Results:** Result of cell migration assay showed that cisplatin at 5, 10 and 20 µM concentrations inhibit GFP-MDA-MB-231 cell migration (Figure S1). Based on the results obtained from this study, we have selected 5 and 10 uM concentrations of cisplatin to test in BBB model.



**Figure S1:** Microscopic images showing the inhibition of GFP-MDA-MB-231 cell migration upon treatment with cisplatin.

***Viability of MDA-MB-231 cells treated with cisplatin***

MTT assay was used to analyze the viability of GFP-MDA-MB-231 cells upon treatment with cisplatin after 5 days of incubation. The cells were seeded in 24 well plates at the density of 50x103 cells/well. After 24 h of cell culture, cisplatin at 5, 10 and 20 µM concentrations were added into the wells. Controls were also maintained. After 5 days of incubation, 50 µl of MTT dye was added according to manufacturer’s protocol (Thermofisher scientific). After 4h incubation, the supernatant was discarded. Then, 200 µl of DMSO (dimethyl sulfoxide) solution was added to dissolve the formazan crystals. Finally, 30 µl of the solution from each well was transferred into 96 well plates. The absorbance was read at 570 nm using Epoch 2 microplate reader. Cell viability was calculated using equation (1).

Cell proliferation (%) = (OD Sample/OD Control) X 100 (1)

All the experiments were repeated 3 times and absorbance were measured in triplicates.