Cross-Disciplinary Optimization of Nano-Drug Delivery to Ovarian Carcinoma and Glioma Cells

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BACKGROUND

Various systems for localized controlled drug delivery to solid tumors have been developed, but the approach still itself remains highly challenging (Bisht and Maitra , 2009). The difficult to control proliferative capacity and invasiveness of solid tumors leads to their spread and metastasis outside of the tumor primary location. Currently used therapies are often limited to the combination of systemic chemotherapy and radiation. In an attempt to improve the outcomes of chemotherapy and limit the toxicity of existing potent anti-cancer agents, numerous research studies have focused on the development of targeted therapies. These approaches involve nano delivery systems in which drugs are being incorporated in variety of vehicles including nano- particles, liposomes, micro-spheres, biodegradable polymers (Wiranowska, et al 1998), and niosomes.

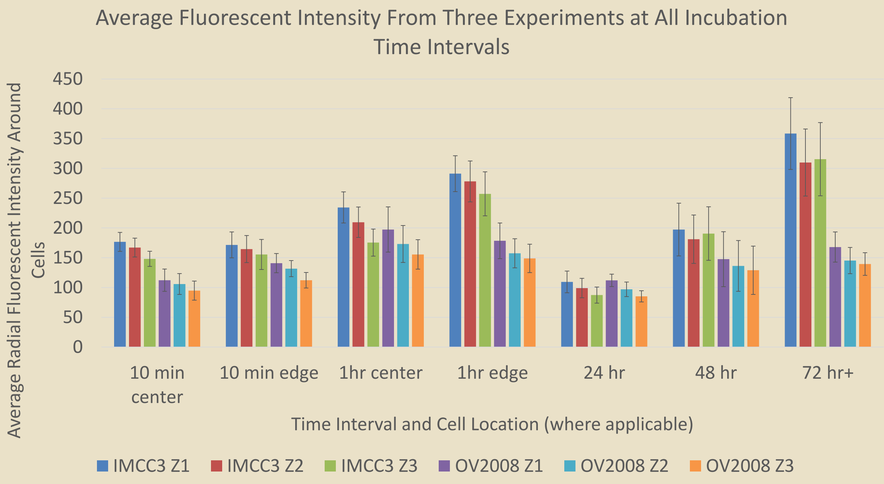
This study reports on the quantitative analysis of the diffusion and localization of a targeted drug delivery system (DDS) consisting of fluorescent labeled 0.01 μM paclitaxel-BODIPY 564/570 encapsulated in non-ionic surfactant vesicles embedded in a thermosensitive cross-linked chitosan hydrogel. This is a multi-tiered DDS that allows for enhanced stability, sustained and controlled delivery of embedded drugs to tumor sites, and reduced toxicity. We showed that cancer cells of epithelial origin, such as human ovarian epithelial carcinoma OV2008 and highly migratory mouse glioma G-26, overexpress MUC1, a mucin surface antigen, which effectively enhances the specific targeting capacity of chitosan. Utilizing this DDS we found that OV2008 carcinoma cells had ~2 times higher fluorescence intensity level than normal ovarian epithelial IMCC3 cells with a statistical significance at 5 min and 24 h incubation times suggesting that this DDS had a higher affinity for tumor cells. Therefore, we hypothesized that there is a difference in PTX diffusion towards epithelial origin tumor cells when compared to normal cells in both distance/location and time.

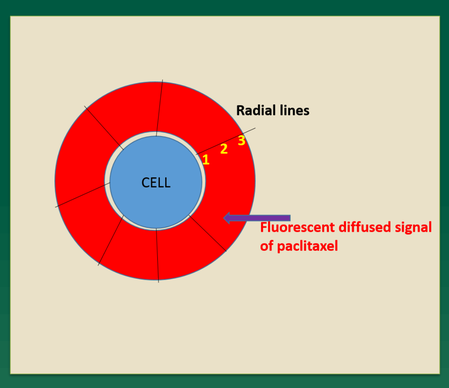
OBJECTIVES

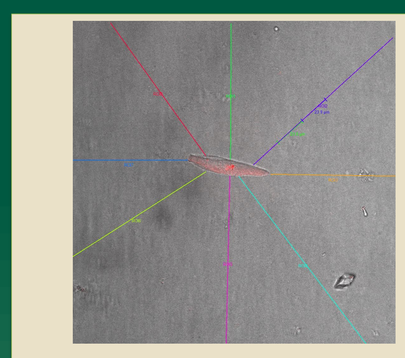
Diffusion and localization of fluorescent labeled PTX was evaluated in vitro using confocal microscopy. The fluorescence intensity captured on the images was quantified with ImageJ software in OV2008 carcinoma cells and compared to IMCC3 normal cells at time intervals 5 min, 1 h, 24 h, 48 h, and 72 h. The fluorescence intensity image data was analyzed in multiple radial line segments separated into three different zones of 24 μm each to represent multiple diffusion distances.

RESULTS

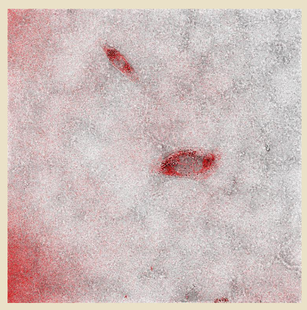
Our results showed that fluorescence intensity levels in the zones around the IMCC3 normal cells at 5 min and 1 h were significantly higher ($p<0.05$) than around OV2008 carcinoma cells correlating reciprocally with our finding of intracellular fluorescence intensity in these two cell lines. Therefore, the data evaluating fluorescence levels in the radial zones outside the cells indicated that the migrating DDS-PTX had already been taken up by the tumor cells. Furthermore, the normal cells which showed significantly lower intracellular levels of fluorescence had higher levels of fluorescence in the measured radial zones outside the cells.



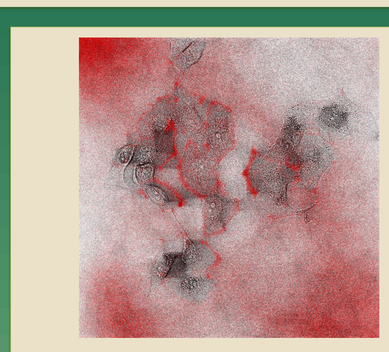




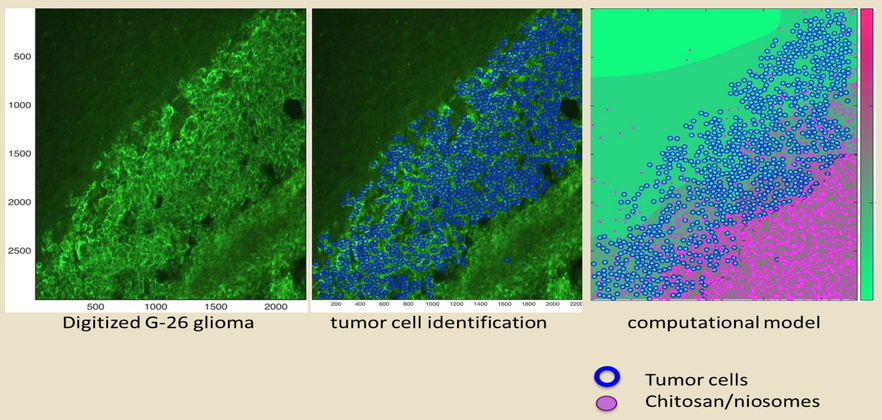
Evaluation and Quantification methodology of Extracellular Intensity



IMMC3 at 24 hrs



Extracellular signal in OV2008 and IMCC3-Images



Future Studies

TABLES

Time Interval	IMMC3	OV2008
10 min center	N =15	N = 8
10 min edge	N = 12	N = 9
1 hr center	N = 10	N = 6
1 hr edge	N = 11	N = 8
24 hr	N = 12	N = 4

Number of extracellular spaces quantified

Time	Slope MCC3	Slope OV2008	R^2 MCC3	R^2 OV2008
10 min center	-14.2	-8.8	-0.98	-0.99
10 min edge	-8.1	-14.4	-1.00	-0.98
1hr center	-29.5	-20.9	-1.00	-1.00
1hr edge	-16.9	-14.8	-0.99	-0.97
24 hr	-11.0	-13.4	-1.00	-1.00
48 hr	-3.4	-9.3	-0.41	-0.99
72 hr+	-21.6	-14.2	-0.81	-0.95

Linear regression data to quantify CTX changes as a unit of distance

Comparison	Significance
IMCC3 Z1 vs OV2008 Z1	P = 0.003
IMCC3 Z3 vs OV2008 Z3	P = 0.001
10 min IMCC3 vs OV2008	P = 0.000006
1 hr IMMC3 vs OV2008	P = 0.00003
24 hr IMMC3 vs OV2008	P = 0.96

T-test Significances

CONCLUSIONS

This PTX-BODIPY 564/570 diffusion data from in vitro studies is currently being used for computational modeling in the in vivo intracerebral model of G-26 glioma. It will be integrated with mathematical model simulations that describe the pharmacokinetic and pharmacodynamic (PK/PD) properties of this drug delivery system for the evaluation of its efficacy and to optimize drug delivery in vivo. Computational modelling is being done in Matlab using the treatment scenario of the post-surgical late-stage glioma. The simulation studies will be used to determine optimal drug concentrations, chitosan density, and localization.

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