Engineering *in vitro* three-dimensional tumor models by surface fabrication

Crystal S. Shin¹, Altug Ozcelikkale², Bumsoo Han², and Kinam Park¹,³

¹Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, Indiana, USA
²School of Mechanical Engineering, Purdue University, West Lafayette, Indiana, 47907, USA
³Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana, 47907, USA
shin16@purdue.edu

ABSTRACT SUMMARY

The *in vitro* cell-based evaluation of anti-cancer drugs is essential to predict *in vivo* therapeutic efficacy. In this study, *in vitro* three-dimensional (3D) tumor models were developed by modifying the surface of a culture vessel. The 3D tumor models were demonstrated as a tool to evaluate cellular drug responses of an anti-cancer drug.

INTRODUCTION

In cancer research, discrepancies in cellular drug responses between cell-based assays and animal/human studies are well recognized. In an effort to minimize these discrepancies, it is necessary to consider a 3D cell culture paradigm in which cancer cells are cultured and allowed to form more physiologically relevant 3D tumors *in vitro*.

Many studies have reported that the 3D tumor models exhibited different morphologies and drug responses (i.e., tumor cells are more resistant to anti-cancer drug in 3D models) in comparison to monolayer models (1,2). Hence, the study of anti-cancer drug responses in a 3D tumor model will be more informative and more clinically relevant.

The objective of our study is to develop a simple, inexpensive, and broadly applicable method to engineer *in vitro* 3D tumor models. We herein present a surface fabrication method using a hydrogel forming biomaterial, gelatin, to promote cellular aggregation. Gelatin is a hydrolyzed form of collagen derived from porcine skin. It is used to promote cell adhesion and widely used in food, biomedical, and pharmaceutical industries (3). Since gelatin as a thermo-sensitive hydrogel is subject to disintegrate at 37°C, cross-linking gelatin is essential to increase its physical and mechanical strength. A naturally occurring cross-linking agent, genipin, is chosen to cross-link gelatin due to its effectiveness in gelatin cross-linking, also its minimal cytotoxicity in comparison to other cross-linkers such as glutaraldehyde (4).

The fabricated surface stimulates the cells to form spherical clusters rather than monolayers. These engineered 3D tumor models are treated with an anti-cancer drug, doxorubicin, to evaluate cellular responses.

EXPERIMENTAL METHODS

Surface fabrication of a culture vessel was achieved by cross-linking gelatin with genipin (Fig. 1A). Type A gelatin was dissolved in phosphate buffered saline and kept at 60°C for 1 hour. A 1% w/w genipin was added to the warm gelatin solution, mixed and transferred into each well of a 96-well plate then allowed to cross-link at 60°C for 4 hours. Cross-linked gels were rinsed with PBS multiple times prior to seeding cells.

In this study, MCF-7 cells, human breast cancer cells, were cultured in RPMI 1640 supplemented with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin. Once MCF-7 cells reached 90% confluency, cells were trypsinized and seeded in wells fabricated with genipin cross-linked gelatin (Fig. 1B). MCF-7 cells were maintained at 37°C with 5% CO₂ and fresh medium was replenished every two days.

To evaluate cytotoxicity of an anti-cancer drug, MCF-7 3D tumor models were treated with Doxorubicin hydrochloride (DOX) for 24 hours. A cytotoxicity assay was performed after the drug treatment using a fluorescence microplate reader.
RESULTS AND DISCUSSION

Gelatin solution upon cross-linking with genipin, forms a blue colored hydrogel. When cells were cultured on uncross-linked gelatin, MCF-7 cells adhered to the plastic bottom of the wells and formed monolayers due to the dissolution of gelatin at 37°C (Fig. 2A). However, the same cells cultured on a gelatin-genipin modified surface formed tightly bound spherical clusters after 5 days (Figs. 2B and 2C).

The drug efficacy study of DOX on MCF-7 cells for 24 hours revealed that a higher concentration of DOX was more effective in causing apoptosis of MCF-7 cells (Fig. 3). The cytotoxicity assay demonstrated that MCF-7 cells in monolayers were more susceptible to the drug resulting in approximately 40% cell death even at 1µM. However, MCF-7 cells in 3D tumor spheroids exhibited higher resistance to DOX when compared with drug responses of MCF-7 monolayers.

CONCLUSION

A simple surface fabrication strategy was developed to engineer in vitro 3D tumor spheroids. It was achieved by cross-linking gelatin with genipin. Seeded MCF-7 cells were induced to form tightly bound 3D tumor spheroids, which displayed higher resistance to the anti-cancer drug, DOX, than the MCF-7 monolayers.

REFERENCES


ACKNOWLEDGMENTS

This study was supported in part by NIH through grants CA129287 and GM095879, and the Showalter Research Trust Fund.