Structure establishment of three-dimensional (3D) cell culture printing model for bladder cancer

[1]Jae Hun Shim, [1]Joon hee Gook, [1]Se Young Choi, [1]Byung Hoon Chi, [1]Jin Wook Kim, [1]Myeong Joo Kim, [2]James J. yoo, [2]Young Min Ju, [1]Tae-Hyoung Kim, [1]Soon Chul Myung, [1]Kyung Do Kim, [1]In Ho Chang

[1]Department of Urology, College of Medicine, Chung-Ang University, Seoul, Republic of Korea
[2]Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA

Abstract

Purpose: Two-dimensional (2D) cell culture is a valuable method for cell-based research but can provide unpredictable, misleading data about *in vivo* responses. In this study, we created a three-dimensional (3D) cell culture environment to mimic tumor characteristics and cell-cell interactions to better characterize the tumor formation response to chemotherapy.

Materials and methods: We fabricated the 3D cell culture samples using a 3D cell bio printer and the bladder cancer cell line 5637. T24 cells were used for 2D cell culture. Then, rapamycin and Bacillus Calmette-Guérin (BCG) were used to examine their cancer inhibition effects using the two bladder cancer cell lines. Cell-cell interaction was measured by measuring e-cadherin and n-cadherin secreted via the epithelial-mesenchymal transition (EMT).

Results: We constructed a 3D cell scaffold using gelatin methacryloyl (GelMA) and compared cell survival in 3D and 2D cell cultures. 3D cell cultures showed higher cancer cell proliferation rates than 2D cell cultures, and the 3D cell culture environment showed higher cell-to-cell interactions through the secretion of E-cadherin and N-cadherin. Assessment of the effects of drugs for bladder cancer such as rapamycin and BCG showed that the effect in the 2D cell culture environment was more exaggerated than that in the 3D cell culture environment.

Conclusions: We fabricated 3D scaffolds with bladder cancer cells using a 3D bio printer, and the 3D scaffolds were similar to bladder cancer tissue. This technique can be used to create a cancer cell-like environment for a drug screening platform.

I. Introduction

- Limitation of two-dimensional (2D) cell culture
  - Almost all cells surrounded by extracellular matrix (ECM)
  - Sometimes provide Unpredictable data *in vivo* response

- Advantage of three-dimensional (3D) model
  - Reproduce the spatial organization and microenvironment factors of *in vivo* micro-tumors
  - Such as, relevant gradients of nutrients and other molecular agents

- 3D bioprinting techniques
  - Make up of cellular material and additives, such as growth factors, signaling moleculars
  - Solve the problem that 3D spheroids models Lack major ECM elements of the tumor microenvironment
  - Offering great potential for regenerative medicine application

- Rapamycin (mTOR inhibitor) : Leading to less control of cancer cell proliferation in unrestrained activation of the PI3K pathway
- Bacillus Calmette-Gue´rin (BCG) : Current bladder cancer treatment
- These medicines effect on drugs differ from actual clinical and laboratory results

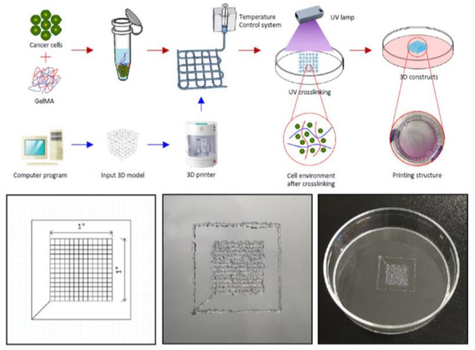
- **Objective**
- ➔ Why the less Effect of rapamycin (mTOR inhibitor) and BCG in the 3D cell culture system?
- ➔ Why the 3D cell culture is more suitable *in vivo* model?

II. Materials & Methods

1. Cells and reagents
   Bladder cell 5637 and T24 cell lines
   Rapamycin and BCG
2. Cell culture and contrast fabrication
   2D cell culture seeded on 60 mm plates, 3D cell culture used by 3D printer
3. Crosslinking conditions setting
   Exposure to UV light (356 nm) using a UV lamp
4. Live/dead staining assay under fluorescence microscopy
5. Cell proliferation assay
   Cell counting Kit-8 was used to analyze cell proliferation
6. Cell viability assay
   After reagent treatment, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done
7. Western blot assay
   Cellect cells after treatment and using a BCA protein Assay Kit
8. ELISA
   Check cytokine (IL-6, IL-12, IFN-γ) level after BCG , Check E-cadherin, N-cadherin expression level
9. Statistical analysis

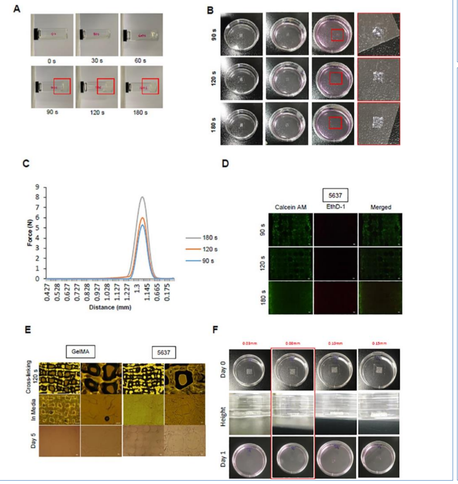
III. Results

Cell culture using simple to complex 3D bioprinting models



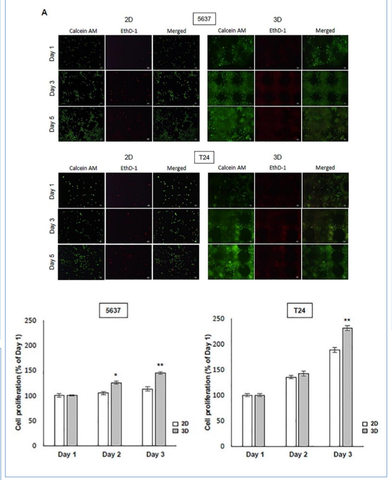
IV. Results

Establishment of the conditions of UV crosslinking and size



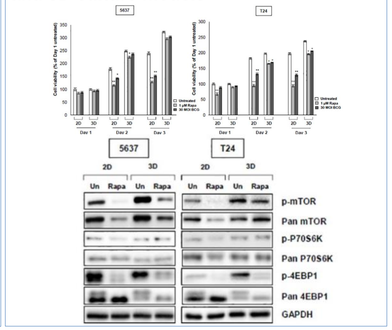
V. Results

Cell survival and proliferation rates in 2D and 3D culture models



VI. Results

The effect of rapamycin and BCG in 2D and 3D culture models

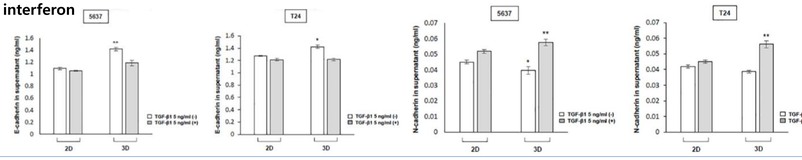


VII. Results

The BCG effect on cytokine production in the 2D and 3D cell culture environment

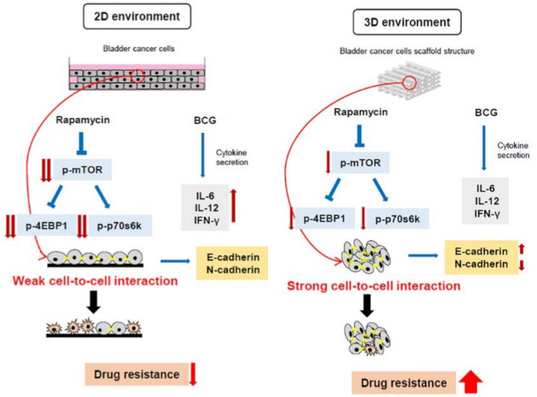
	5637				T24			
	2D		3D		2D		3D	
	untreated	BCG	untreated	BCG	untreated	BCG	untreated	BCG
Cytokine level (pg/mL, mean ± SD)								
IL-6	195.29 ± 2.30	220.66 ± 2.12**	190.74 ± 2.42	198.45 ± 1.61	162.69 ± 4.81	274.31 ± 6.05**	128.94 ± 4.66	195.21 ± 11.58*
IL-12	29.62 ± 0.66	46.75 ± 0.45**	25.52 ± 0.99	37.44 ± 1.50*	45.38 ± 1.36	60.86 ± 1.77*	29.33 ± 0.44	34.65 ± 1.85
INF-γ	33.43 ± 1.00	40.69 ± 1.05**	34.50 ± 0.54	36.57 ± 1.11	46.00 ± 0.23	51.54 ± 0.19**	42.34 ± 0.97	44.92 ± 0.34

p<0.05 and
** p<0.01
IL: interleukin, INF: interferon



VIII. Discussion

Hypothetical schema of comparison of 2D and 3D environments due to cell-to-cell interaction



IX. Conclusions

- Effect of drugs (Rapamycin and BCG)
  ➔ More exaggerated in the 2D cell culture environment
  ➔ The difference in drug resistance according to the difference in the intensity of cell-to-cell interaction
  ➔ This explains why rapamycin and BCG have shown excellent efficacy in research studies but not in clinical studies and patients.

- 3D cell culture advantage
  1) Faster and stable
  2) Higher drug resistance and less sensitivity
  3) More similar to the cell-to-cell interaction and basal action observed *in vivo* environment
  -> personalized medicines for more accurate predictions of specific personal responses to drugs

- 3D bladder cancer cell culture
  -> Similar to bladder cancer tissue
  -> Used to create a cancer cell-like environment for a drug screening platform