A NOVEL PLATFORM FOR ENGINEERING THE TUMOR 3D MICROENVIRONMENT

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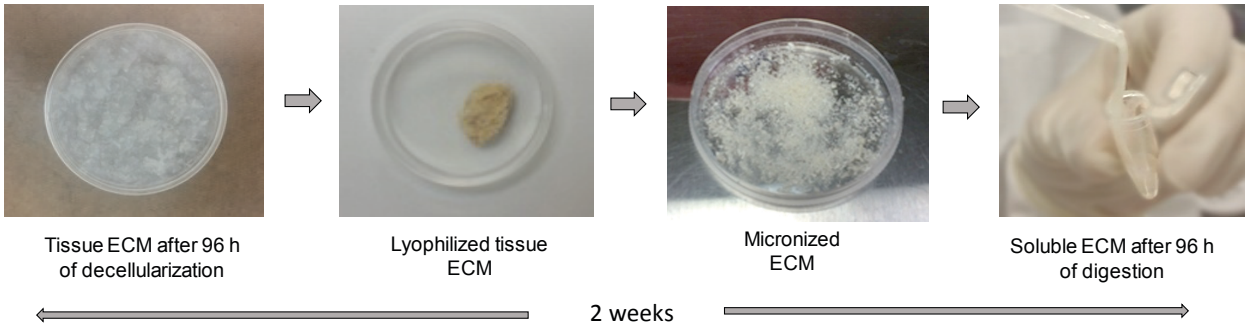
INTRODUCTION

Cancer research traditionally relies on 2D monolayer cell cultures, non-physiological systems that expose cells to inappropriately stiff surfaces and fail to reproduce in vivo cell interactions. Improving in vitro anti-cancer drug testing is possible by mimicking the cell microenvironment through engineering of the surrounding extracellular matrix (ECM). Compared to 2D, an ECM-based 3D model more closely resembles a tissue's physiological and pathological conditions. Compared to normal ECM, the stroma of invasive tumors becomes enriched in dense fibrillar collagen as a result of the desmoplastic reaction. Specifically, glioma cells were shown to modify local microenvironment by releasing highly rigid ECM. Fibronectin, laminin and collagens were shown to have stimulatory effects on glioma cell migration. Currently available commercial ECM products use xenograft or synthetic materials that may not be entirely compatible with human tissue and are not specialized for specific tissue types.



AIM

The study goals were to develop LatBioGel, a 100% human allograft ECM-based product that mimics the cell microenvironment, and to test LatBioGel's suitability for 3D culturing of the glioblastoma cell line U-87. Furthermore, we explored different tissue and cell culture sources for the presence of glioma cell-stimulating ECM components in order to further customize LatBioGel properties for brain tumor research.

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CELL CULTURE & ECM ISOLATION

Adipose-derived stem cells (ADSCs) were isolated from cadaveric human adipose tissue and cultured to acquire soluble secreted ECM. Soluble ECM was purified and concentrated by centrifugation using Amicon 10kDa fmembrane microconcentrators. Insoluble ECM was collected by decellularization of confluent tissue culture cells and scraping adherent ECM.

ECM was isolated from human calf muscle and placenta by decellularization followed by lyophilization. Lyophilized ECM was milled to a fine powder and subjected to enzymatic digestion. LatBioGel was generated by combining ADSC and tissue ECMs according to a proprietary process

ECM ANALYSIS

To evaluate DNA content, total DNA was isolated from 25 mg of ECM and measured by absorption at 260 nm. Total protein amount in ECM was evaluated using the BCA protein assay followed by absorbance measurement at 562 nm. western blot analysis was conducted on loads 25 µg of ECM.

Protein expression in ECM generated from human placenta was measured using Human Cytokine Array C1000 (RayBiotech). This Cytokine Array utilizes the sandwich immunoassay principle, where a panel of capture antibodies is printed on a nitrocellulose membrane solid support. The array membranes are processed similarly to a western blot (chemiluminescent readout). Signals are then visualized on x-ray film or a digital image, allowing densitometry data collection and calculation of fold-changes for each detected protein.

FUNCTIONAL ECM ASSESSMENT

Proliferation assay: 96-well cell culture plates were thin-coated with LatBioGel or Matrigel (Corning). Uncoated wells were used as a control. To generate a thick gel, Matrigel or LatBioGel (>5 mg/ml) were added to each well and gelation allowed at 37°C. U87 cells were plated at a density of 5,000 cells per well. Cells were incubated with MTT reagent for 4 h at 37°C. Formazan was solubilized and its concentration was determined by optical density measurement at 570 nm.

Live/Dead Viability/Cytotoxicity Assay:
24-well cell culture plates were coated with LatBioGel or Matrigel as described above. U-87 cells were seeded at the density of 10,000 cells/well. Cells were labeled with Hoechst 33342 and Propidium Iodide. Fluorescent imaging was performed using Nikon Diaphot microscope. Total cell number was estimated using 360/460 nm filter and dead cells were detected using 535/617 nm filter. Three images per condition were taken. The percentage of dead cells was calculated using Image J software.

RESULTS

Biochemical analysis demonstrated a lack of trace DNA in the ECM indicating complete removal of cellular components (Fig. 1). LatBioGel was characterized for presence of soluble collagen, sulfated GAG, and protein content (Fig. 2). Stability studies show that 3D LatBioGel is stable for at least 14 days at 37C. Proteomic analysis showed the expression of 38 cytokines and growth factors, including those involved in matrix remodeling, angiogenesis, cell growth and differentiation (Fig. 3). In order to further customize LatBioGel for the glioma-specific microenvironment, ECMs were isolated from various tissue and cell culture sources and analyzed for the presence of glioma-stimulating ECM components such as collagens, laminin, and fibronectin (Fig. 4).

To demonstrate that LatBioGel is suitable for culturing brain tumor cells, we utilized U-87MG (Human glioblastoma-astrocytoma, epithelial-like cell line, ATCC). As a control, 2D culture and Matrigel 3D culture were utilized. Thin tissue plate coating and 3D gel formation were tested. Uncoated tissue culture plates and xenograft-based coating (Matrigel) were used as controls. We observed less cell spreading and differences in cell morphology in U-87 cells cultured on LatBioGel compared to controls (Fig. 5).

Functional assays, such as live/dead assays and the MTT proliferation test, revealed that LatBioGel supports cell growth (Fig. 6) and proliferation (Fig. 7).

CONCLUSION

LatBioGel can provide a suitable microenvironment for preserving tumor cell viability and thus should be ideal for creating 3D cell culture models to test cancer cell drug response in vitro. Future efforts will involve further customization of LatBioGel utilizing other novel ECM components to achieve improved performance and better recapitulation of a tissue-specific microenvironment.

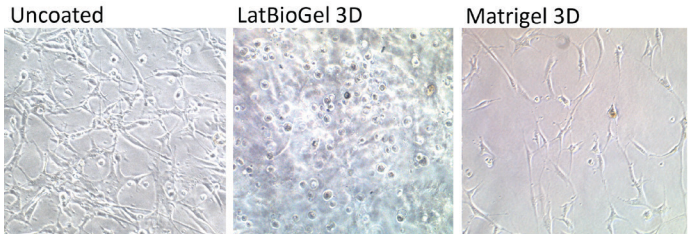


Figure 5. Spreading and morphology of glioblastoma cancer cells were dramatically altered upon 3D culture on LatBioGel

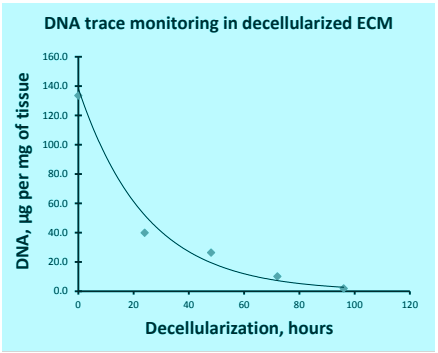


Figure 1. ECM decellularization was monitored by measuring the traces of DNA

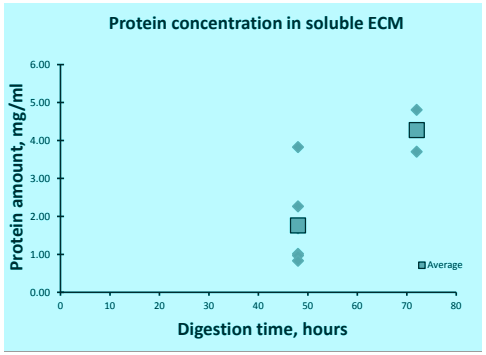


Figure 2. Protein concentration in ECM correlates with digestion time

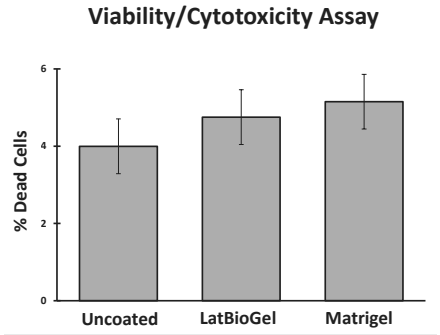


Figure 6. LatBioGel supports viability of U-87 glioblastoma cells

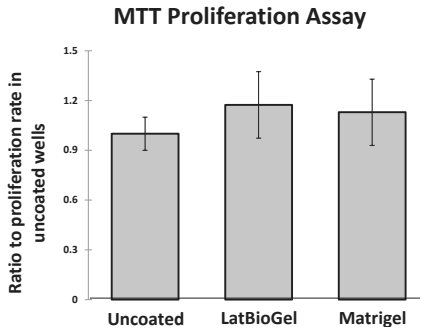


Figure 7. LatBioGel supports proliferation of U-87 glioblastoma cell line

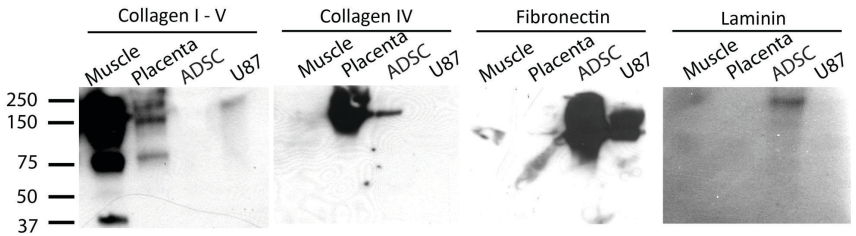
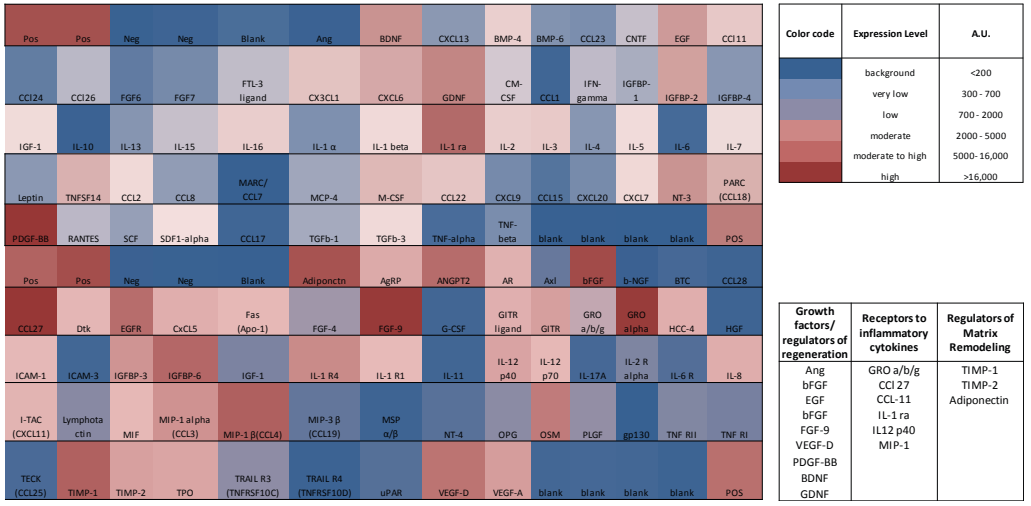


Figure 4. LatBioGel properties can be further customized by combining ECM components of different sources. Western Blot analysis demonstrated differences in ECM composition depending on tissue source. ECM was isolated from human muscle, placenta, adipose-derived stem cells (ADSC) and glioblastoma cell line (U-87)



Growth factors/ regulators of regeneration	Receptors to inflammatory cytokines	Regulators of Matrix Remodeling
Ang	GRO a/b/g	TIMP-1
bFGF	CCI 27	TIMP-2
EGF	CCL-11	Adiponectin
bFGF	IL-1 ra	
FGF-9	IL12 p40	
VEGF-D	MIP-1	
PDGF-BB		
BDNF		
GDNF		

Figure 3. Protein expression in LatBioGel was measured using Human Cytokine Array C1000 (RayBiotech) shows relative expressions of proteins ranging from low or background level (blue color) to high level (red color). Table (bottom right) highlights some of the highly expressed cytokines in placental ECM that play a role in regulation of regeneration, inflammation, and matrix remodeling