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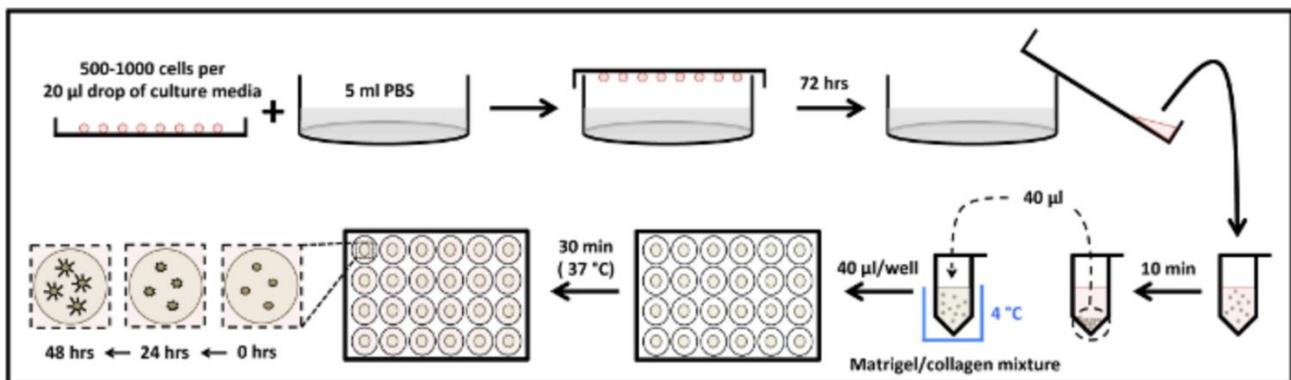
OviGenex Collagen Test

This test compared the performance of OviGenex collagen with Vendor Type I collagen in a three-dimensional (3D) cell invasion assay.

Methods

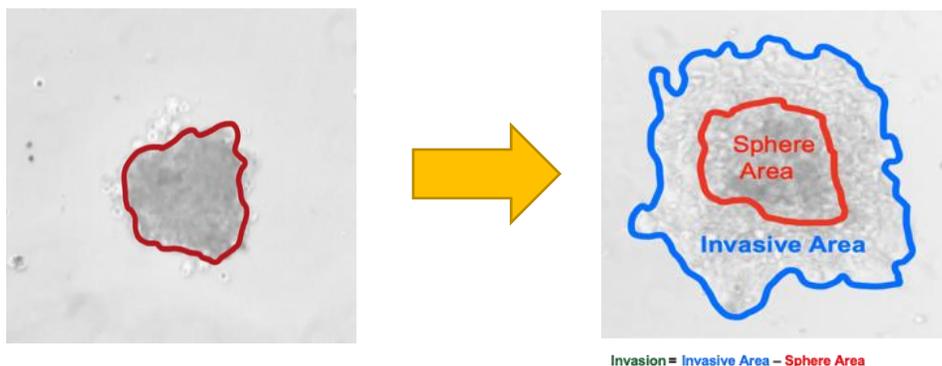
The 3D cell invasion assay was performed based on the method described by Berens et al. (*J. Vis. Exp.* (105), e53409, doi:10.3791/53409 (2015)). The BT549 breast cancer cells (ATCC HTB-122) that are highly invasive were used in the assay. The 3D matrix used in the invasion assay was prepared by mixing either OviGenex collagen from Molecular Express or Type I collagen (rat tail) from Vendor with Corning Matrigel GFR Membrane Matrix (Corning, Cat: 356230). While the Vendor collagen was used at 2 mg/mL final concentration as recommended by the manufacturer, OviGenex collagen was used at various concentrations. The cell spheres were imbedded in the 3D matrix and the invasiveness of the cells was analyzed by measuring the expansion of cell front lines. The diagram below illustrates the experimental procedure.

3D invasion assay



Result Analysis

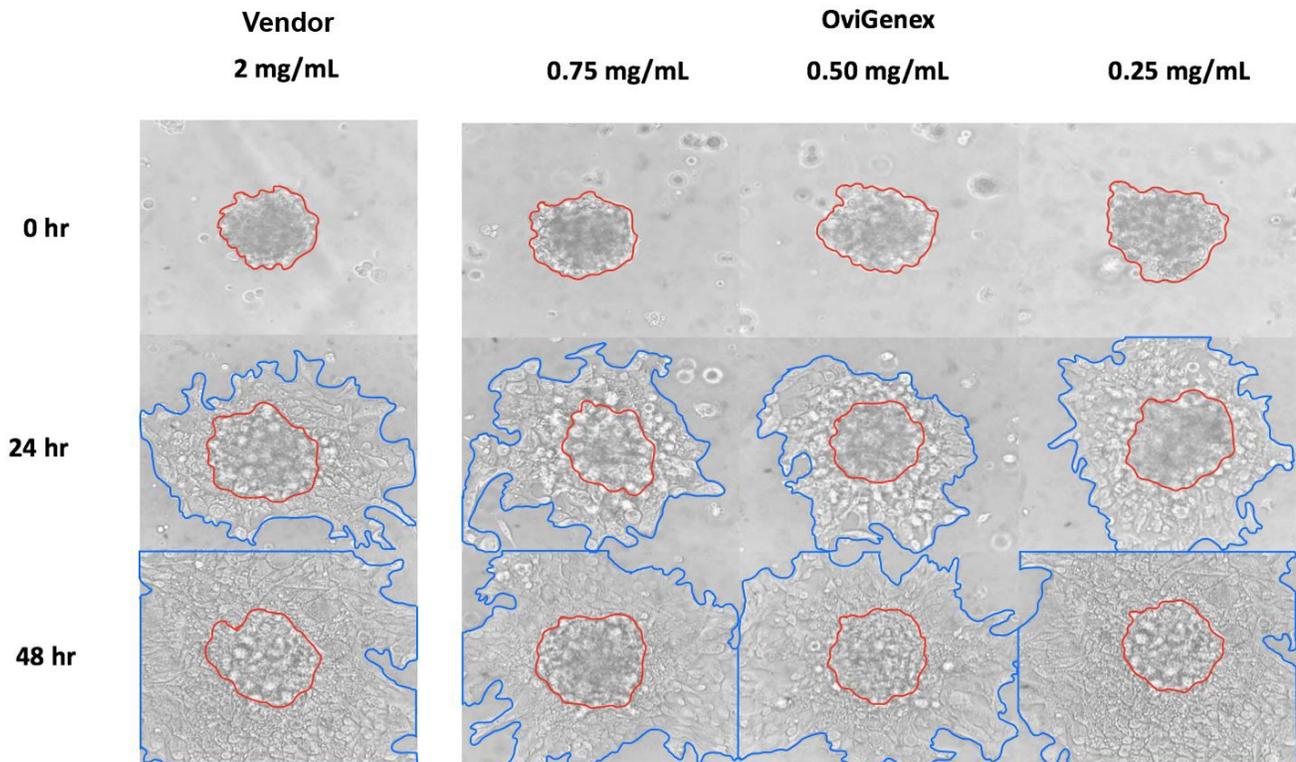
The invasiveness of the BT549 cells were observed 24 hours or 48 hours after the spheroids were plated. The invasiveness of each spheroid was determined by the distance of the invasive cell front line (blue) from the original sphere area (red).



Results

Vendor collagen was used at a final concentration of 2 mg/mL in the 3D matrix as suggested by the manufacturer, and significant cell invasion was observed (left column). However, we found that at 2 mg/mL, the OviGenex collagen was very viscous and the matrix slowed down the invasion of the same cancer cells and resulted in a significant smaller invasion area when compared to cells in the Vendor collagen-based 3D matrix. Therefore, we diluted OviGenex collagen to 0.75, 0.5 and 0.25 mg/mL, and found that at 0.25 mg/mL, the OviGenex collagen was still able to give a result similar to that in Vendor collagen-based matrix, i.e. the sizes of the invasion area were similar. We also noticed that when mixing the collagen with the Matrigel, the 0.25 mg/mL OviGenex had very similar viscosity in comparison to the Vendor collagen. We thus concluded that a similar 3D invasion assay result could be obtained from 3D matrix prepared with OviGenex collagen at a much lower concentration (0.25 mg/mL) when compared to Vendor Type I collagen, which was used at a concentration of 2 mg/mL.

Collagen Concentration Comparison



Junjun Liu, Ph.D.
Professor
Department of Biological Sciences
California State Polytechnic University, Pomona
3801 W Temple Ave., Pomona, CA 91768