Modeling Human Gliomas *In Vitro*

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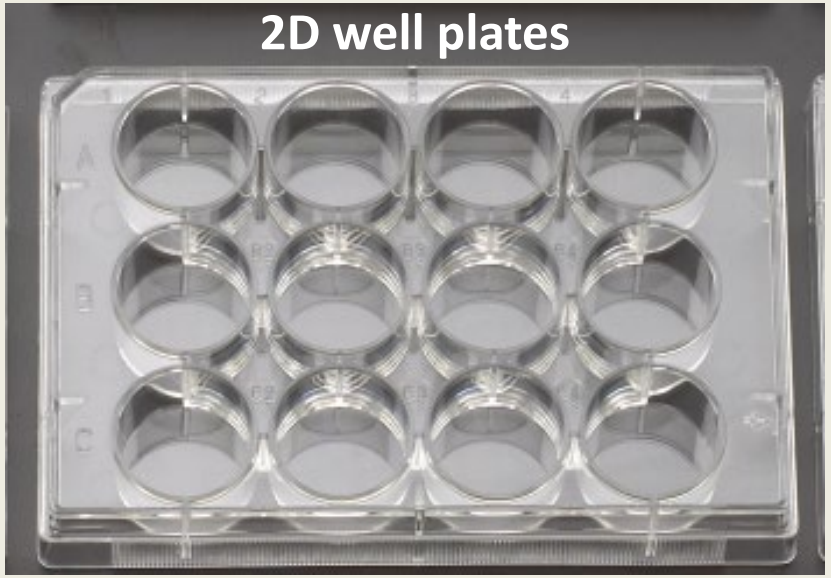
In vitro to *in vivo*

- A need for better *in vitro* models that mirror *in vivo* environments of gliomas
- Difficulties with transitioning from *in vitro* 2D models to *in vivo*
- 3D Chitosan-alginate (CA) scaffolds were tested and found to support cell growth that more closely matches *in vivo* tumors due to their porous properties
- Cells grown in CA scaffolds manifest a more malignant phenotype and develop higher cancer stem cell characteristics than those grown in 2D plates

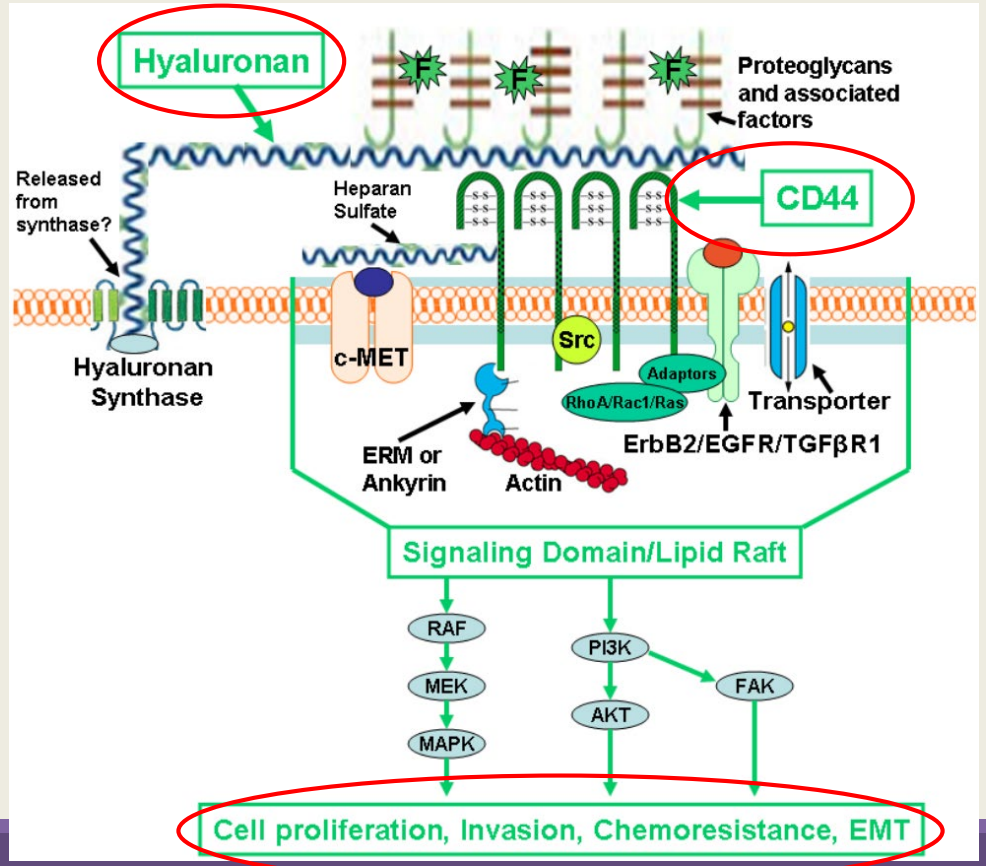


Miqin Zhang, University of Washington

Objective

- To determine cell growth and behavior in 2D plates versus 3D **chitosan hyaluronic acid (CHA)** scaffolds with fluorescent imaging and analysis of differences in protein expression of U-87 human glioma cells
- Hyaluronic acid is part of the extracellular environment of tumors
- Target proteins that will be analyzed are the following:
  - **CD44** is a glycoprotein that plays a role in metastasis, adhesion, and angiogenesis. It's also a receptor for hyaluronic acid that signals pathways affecting tumor progression and invasion.
  - **Nestin** is a protein associated with malignancy and serves as a marker for cancer stem cells

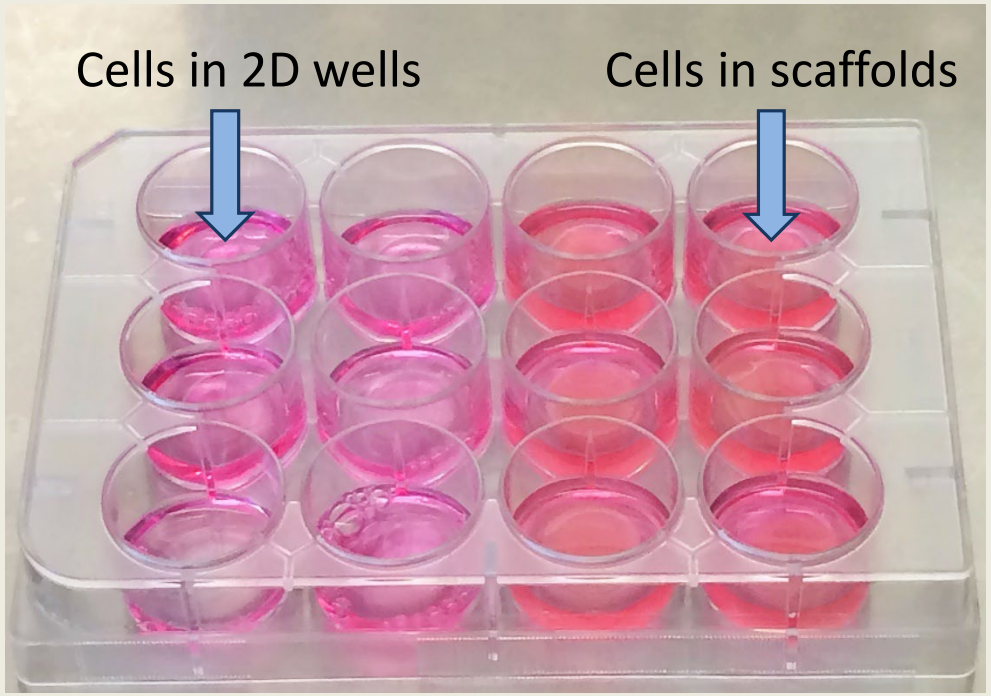
Wang, *Biomaterials*



Toole, *Clin Cancer Res.*

Process

- Culture U-87 cell line in both 2D well plates and 3D CHA scaffolds
- Image cells over a 10 day period to observe cell growth in the two environments
- Determine proteins present and relative quantities by running Western Blot



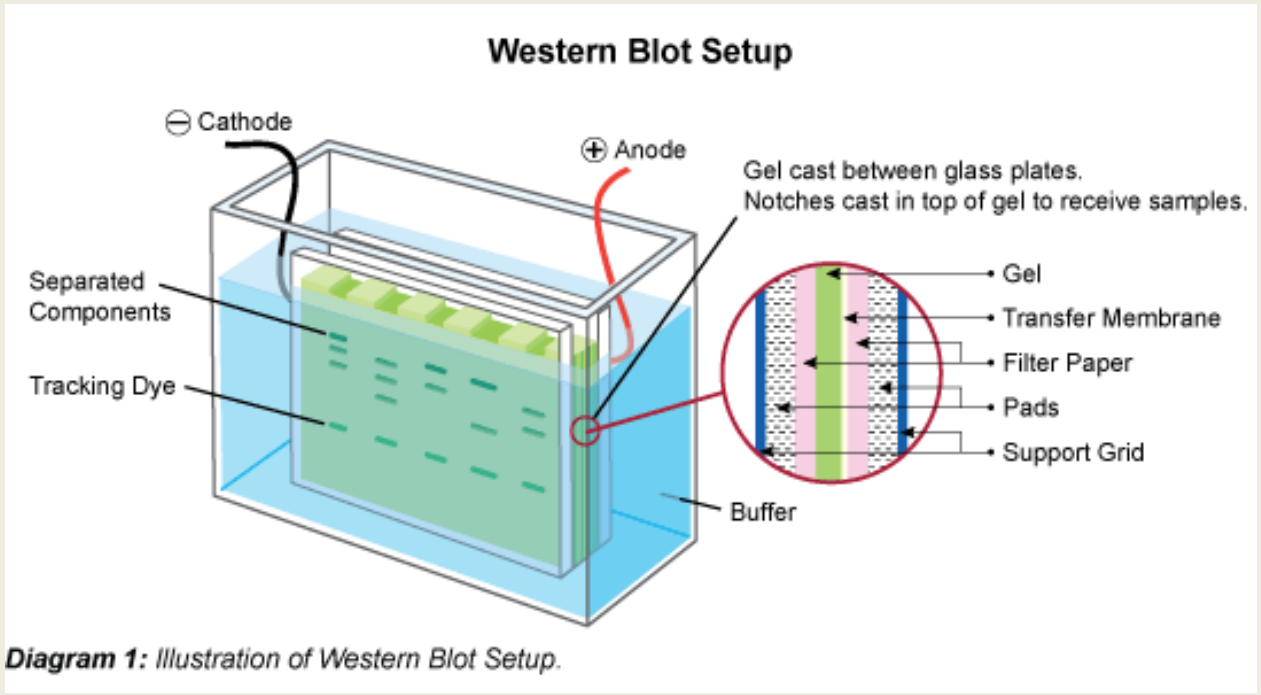
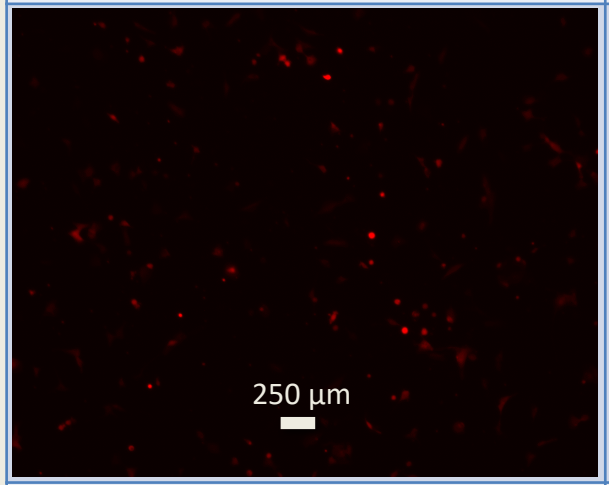
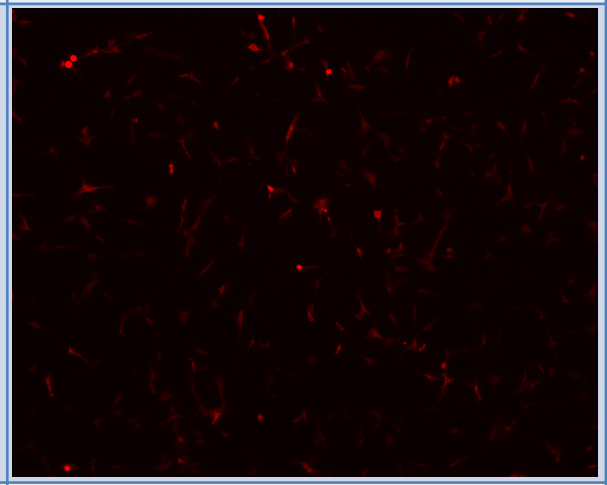
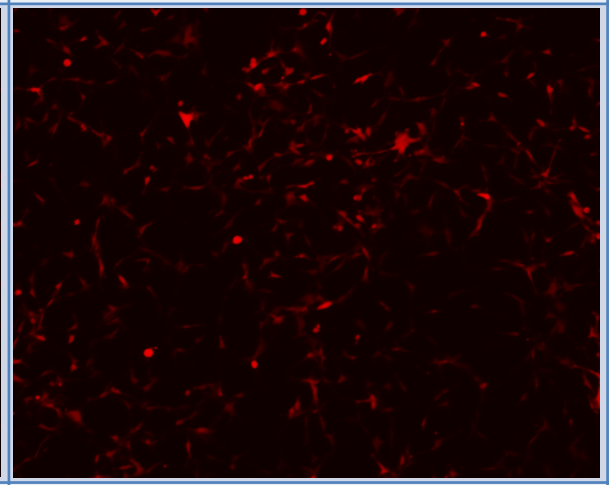
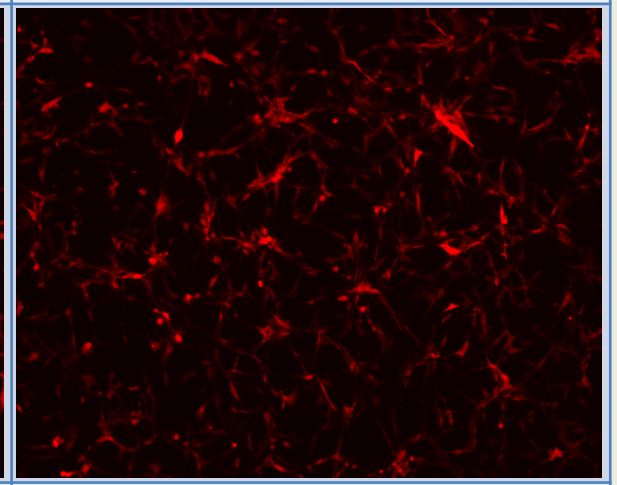
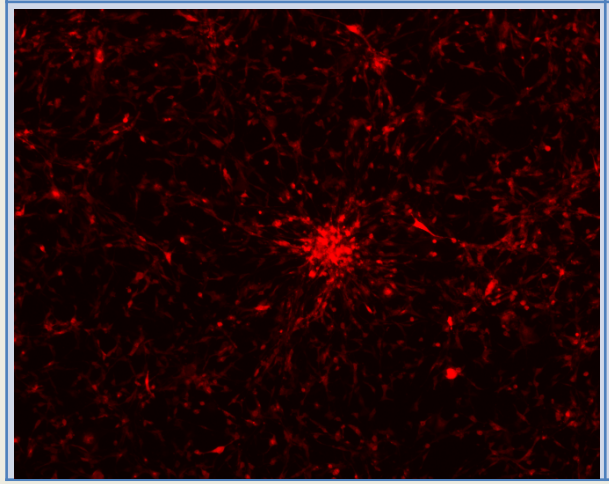
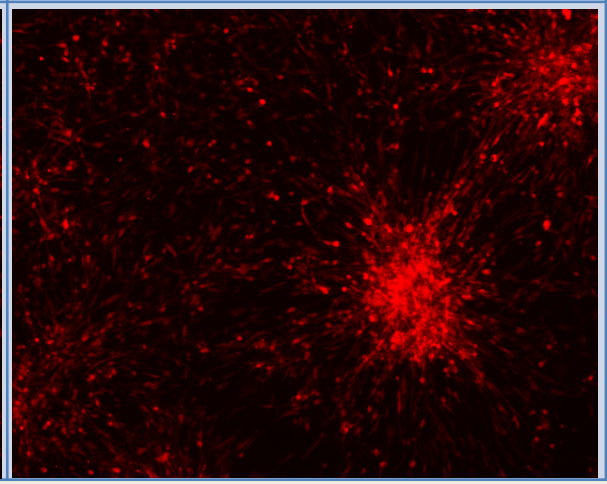
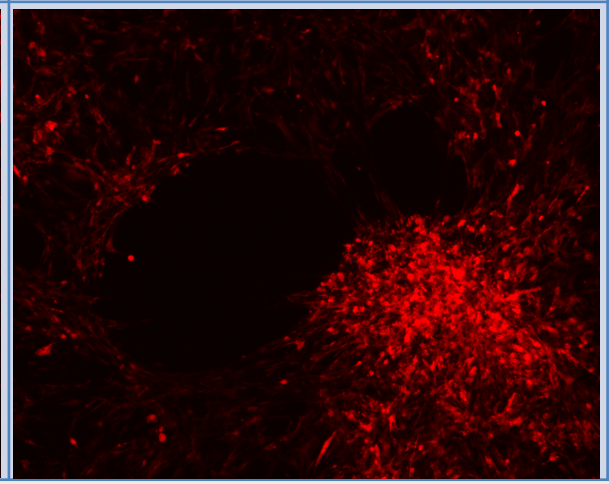
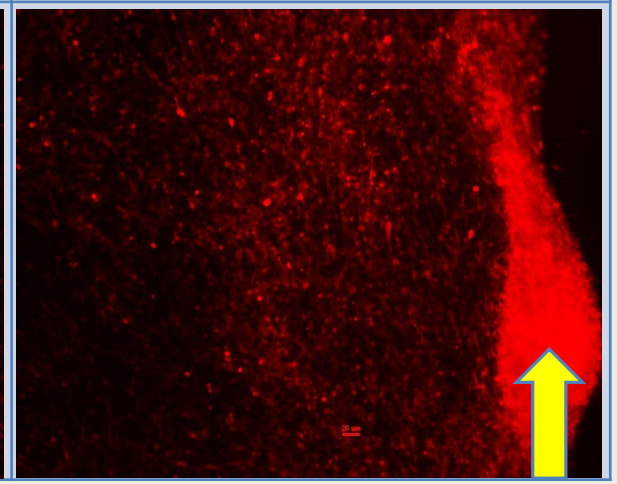


Diagram 1: *Illustration of Western Blot Setup.*

4X: U-87 cells in 2D plates

Day 1	Day 2	Day 3	Day 4
			
Day 7	**Day 8**	**Day 9**	**Day 10**
			

*cells on the edge of well plate overgrow and become confluent

20X: U-87 cells in 2D plates

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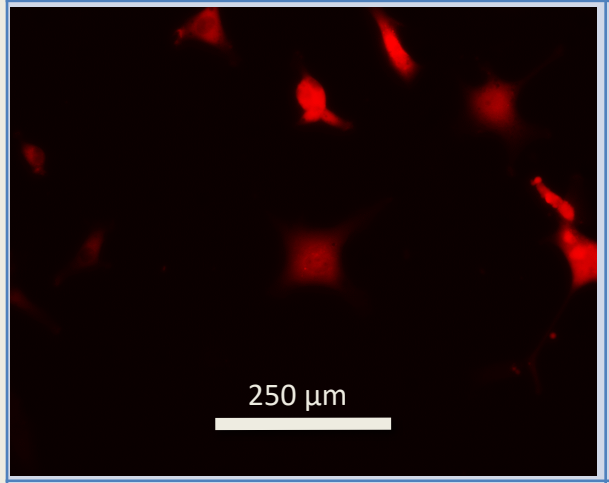	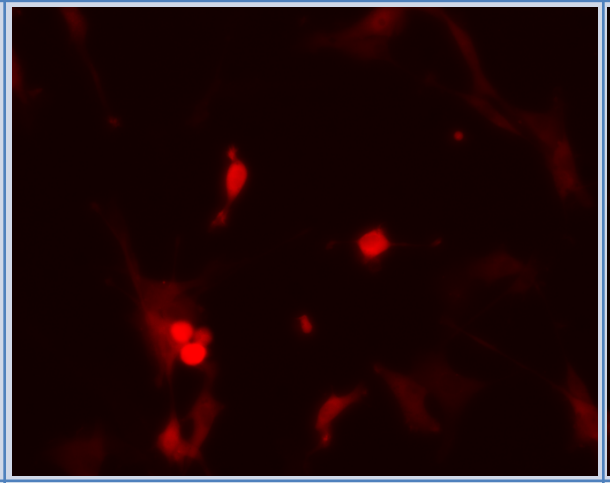	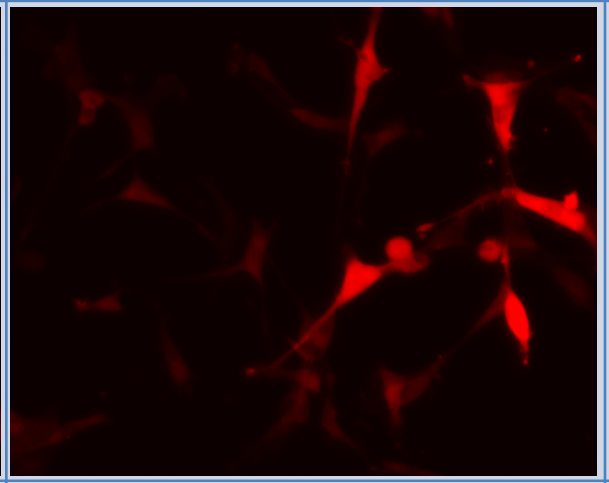	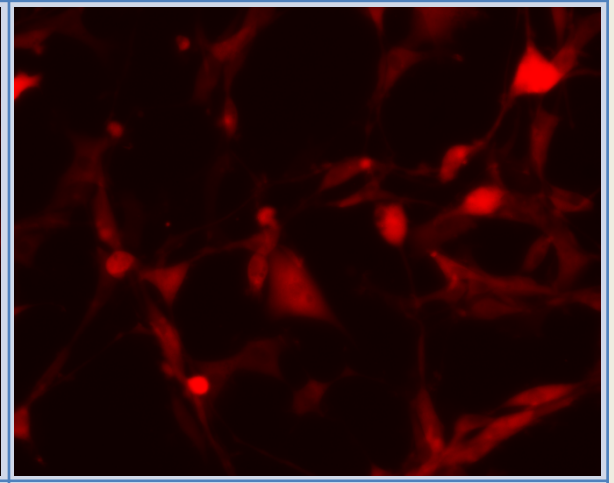
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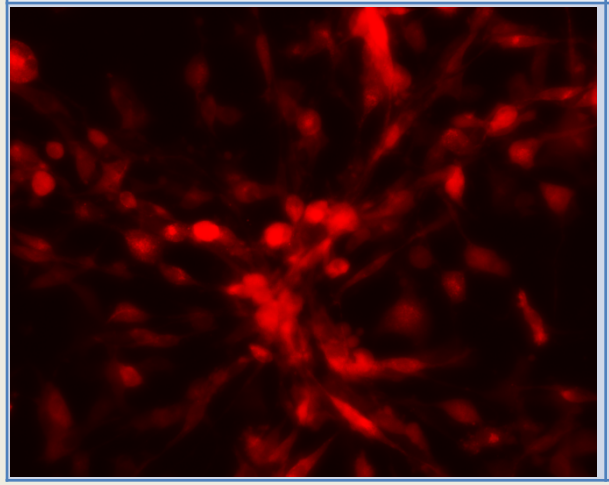
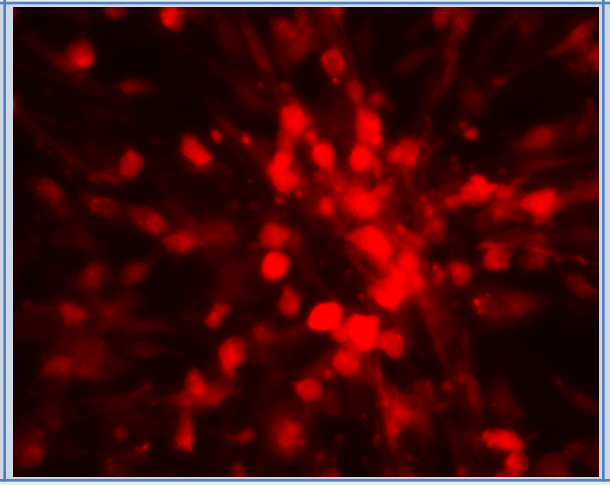
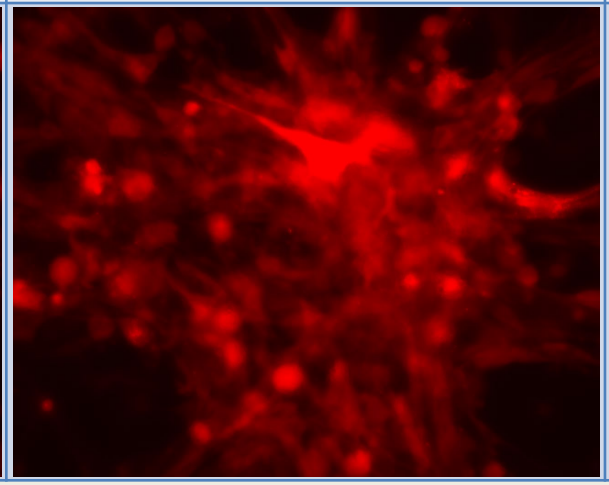
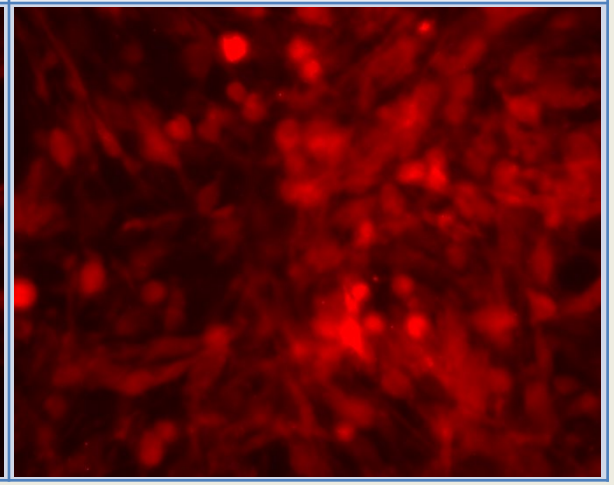
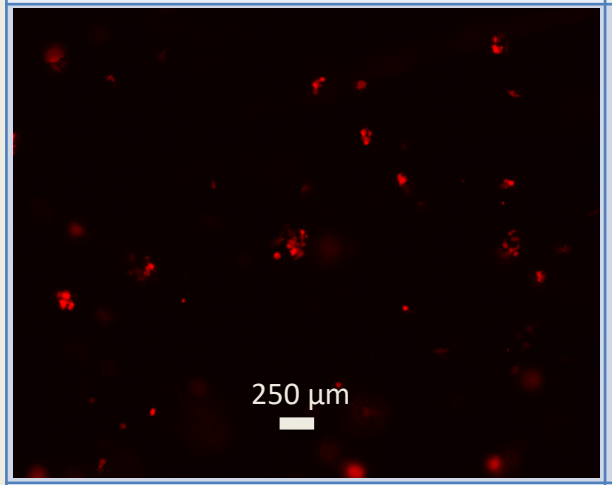
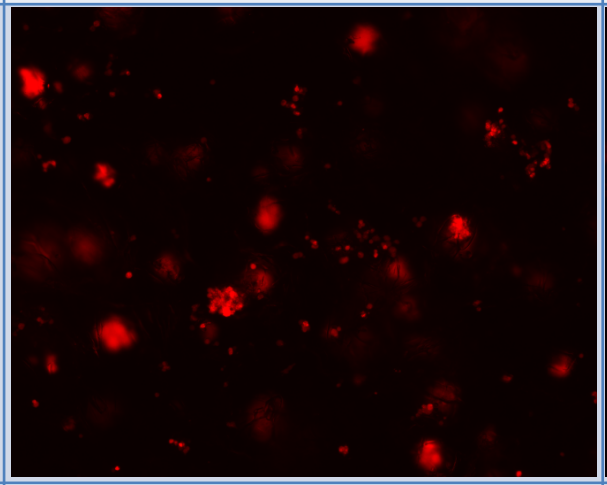
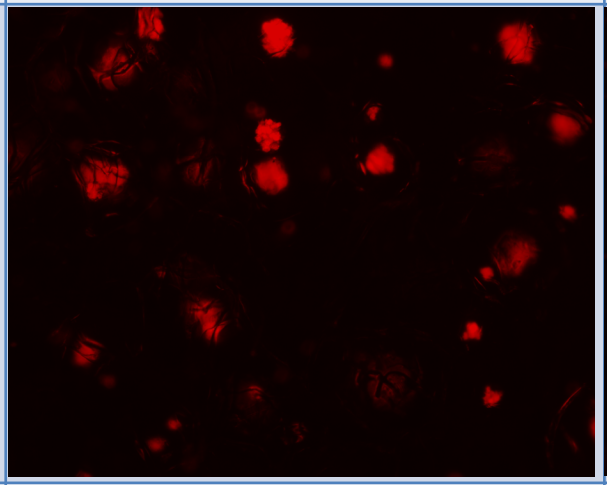
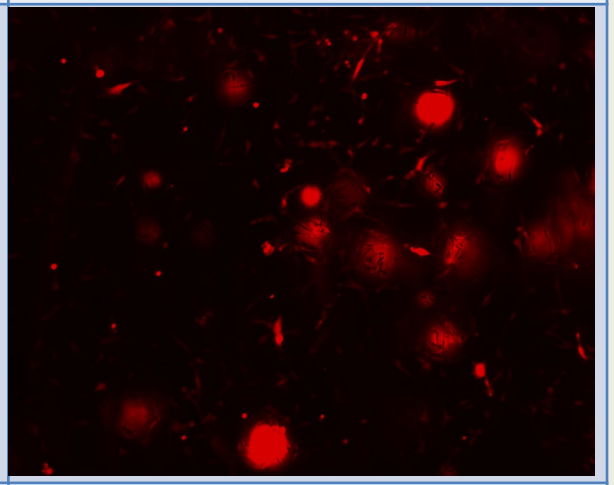
			

4X: U-87 cells in CHA scaffolds

Day 1	Day 2	Day 3	Day 4
			
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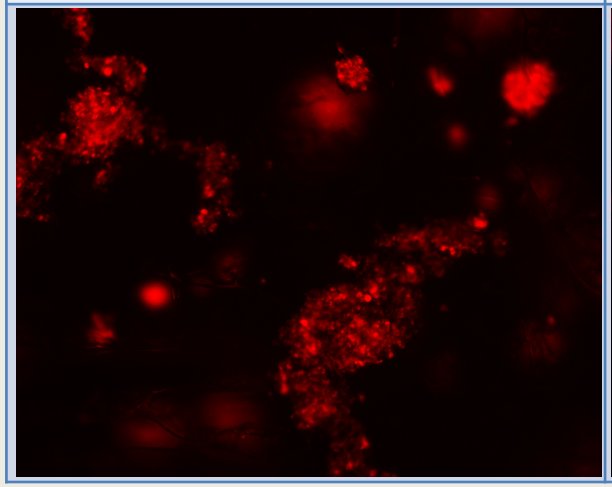
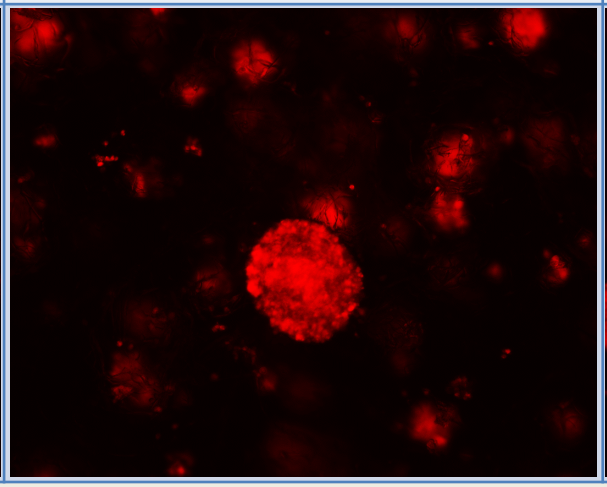
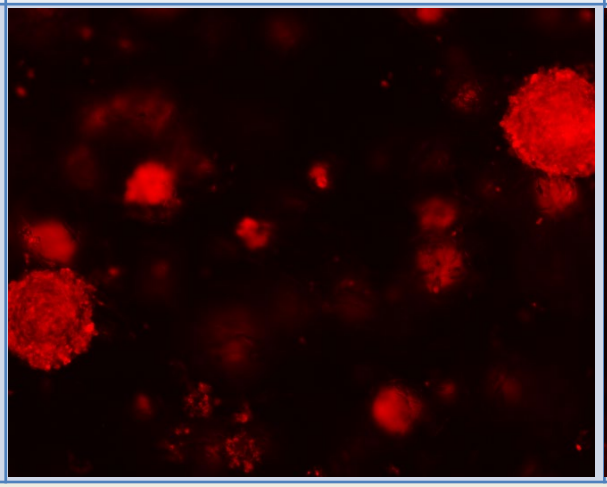
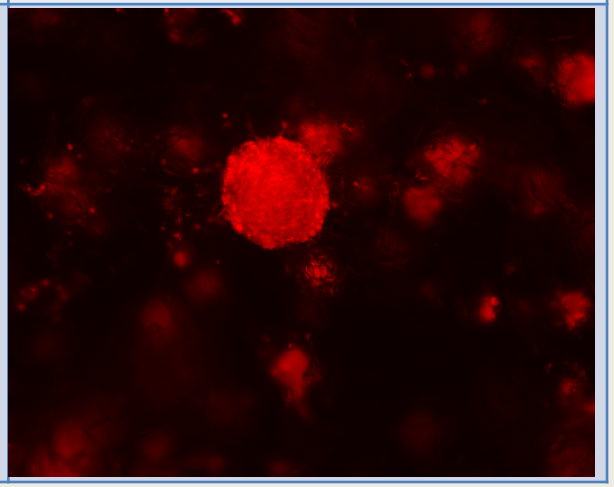
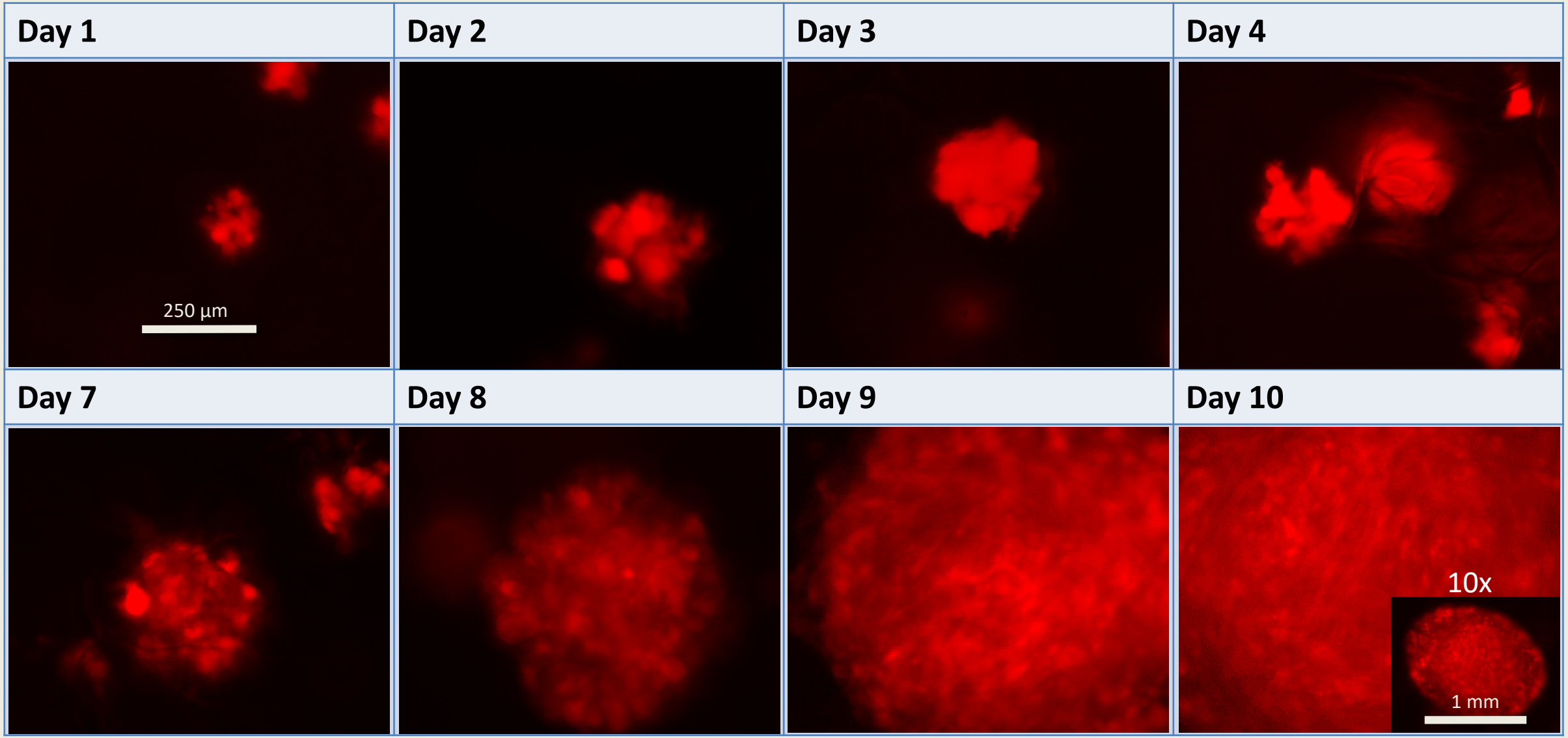
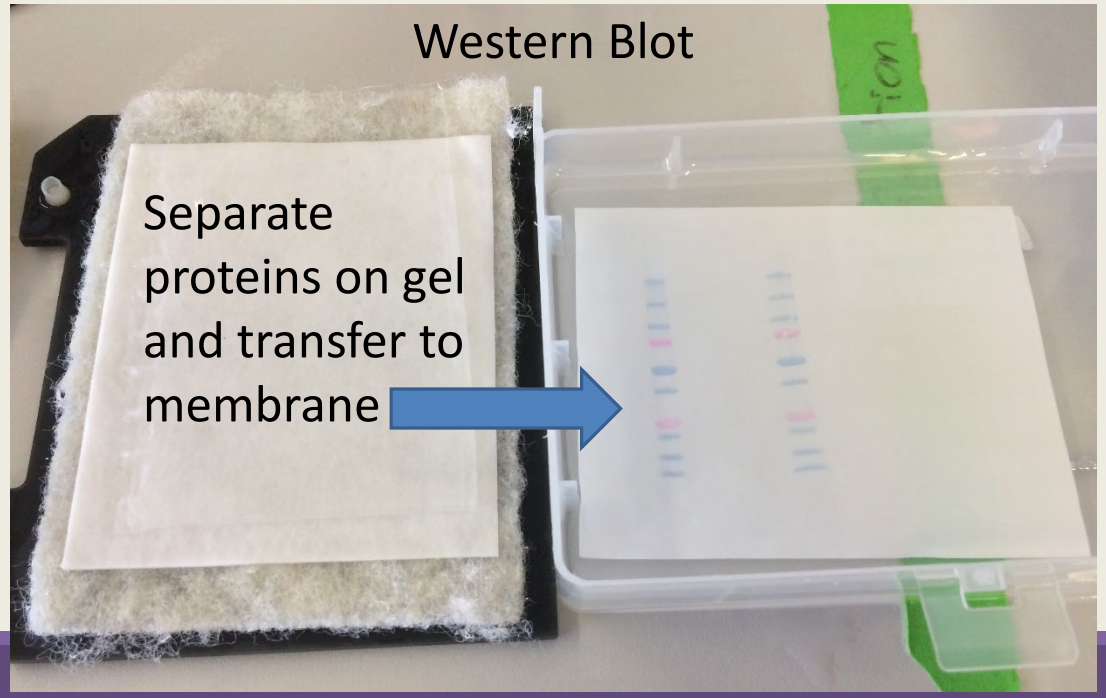
			

20X: U-87 cells in CHA scaffolds



Western Blot

- Lyse cells to release proteins
- Separate proteins through electrophoresis
- Transfer proteins onto a membrane
- Probe protein of interest with antibodies
- Develop membrane with chemiluminescent substrate
- The light is detected and is used to compare quantity of protein present



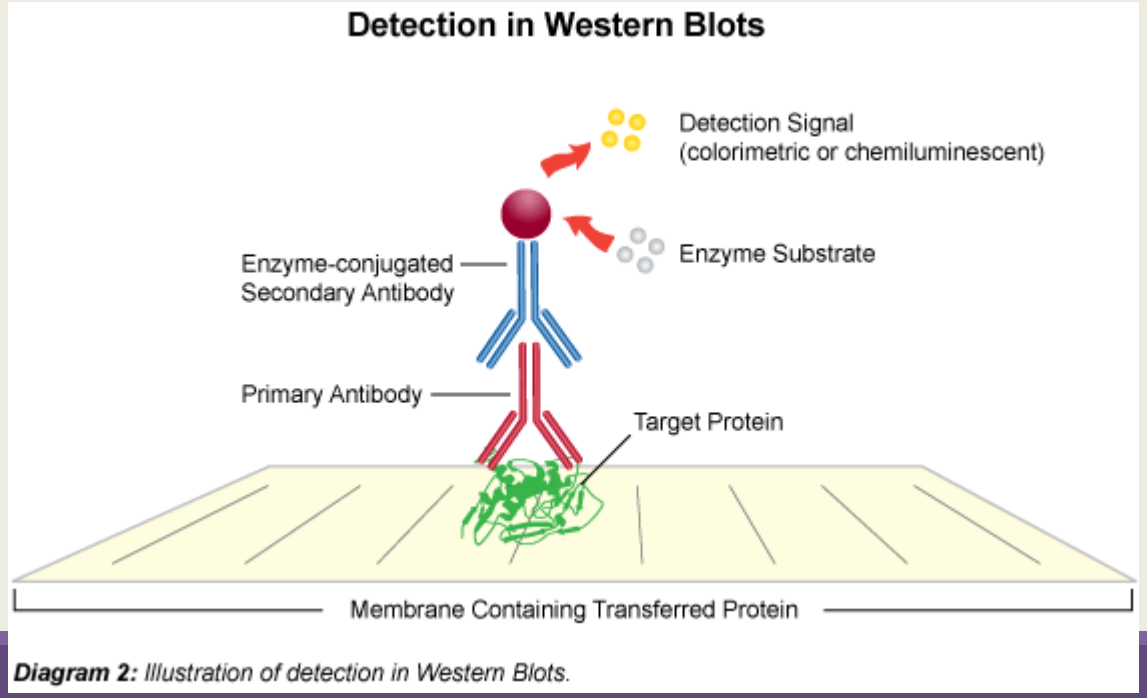
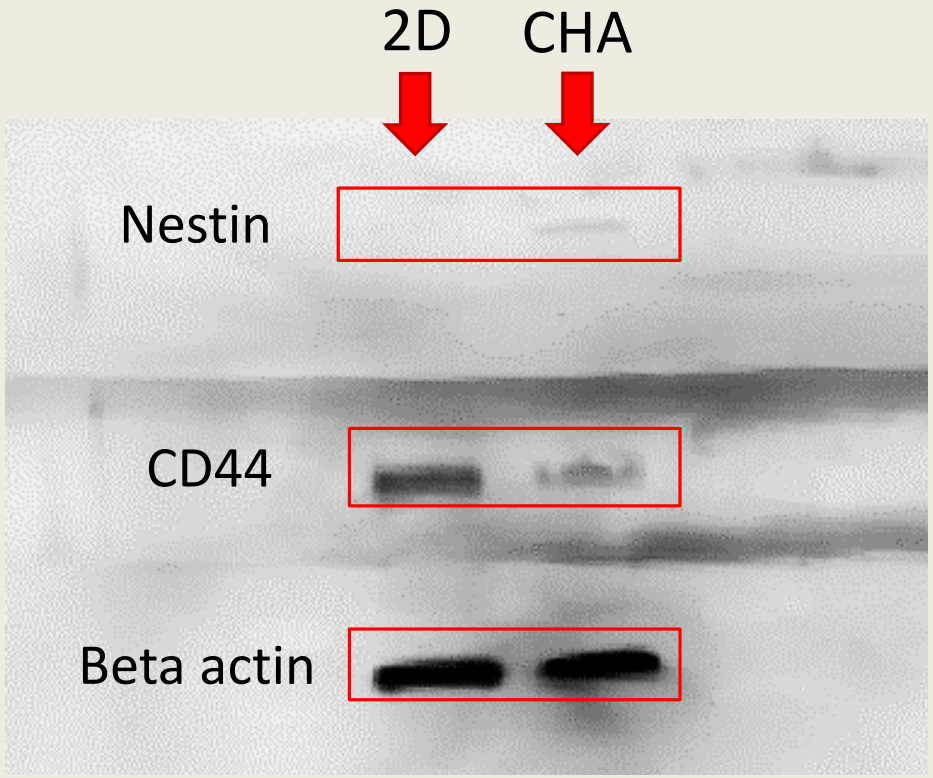
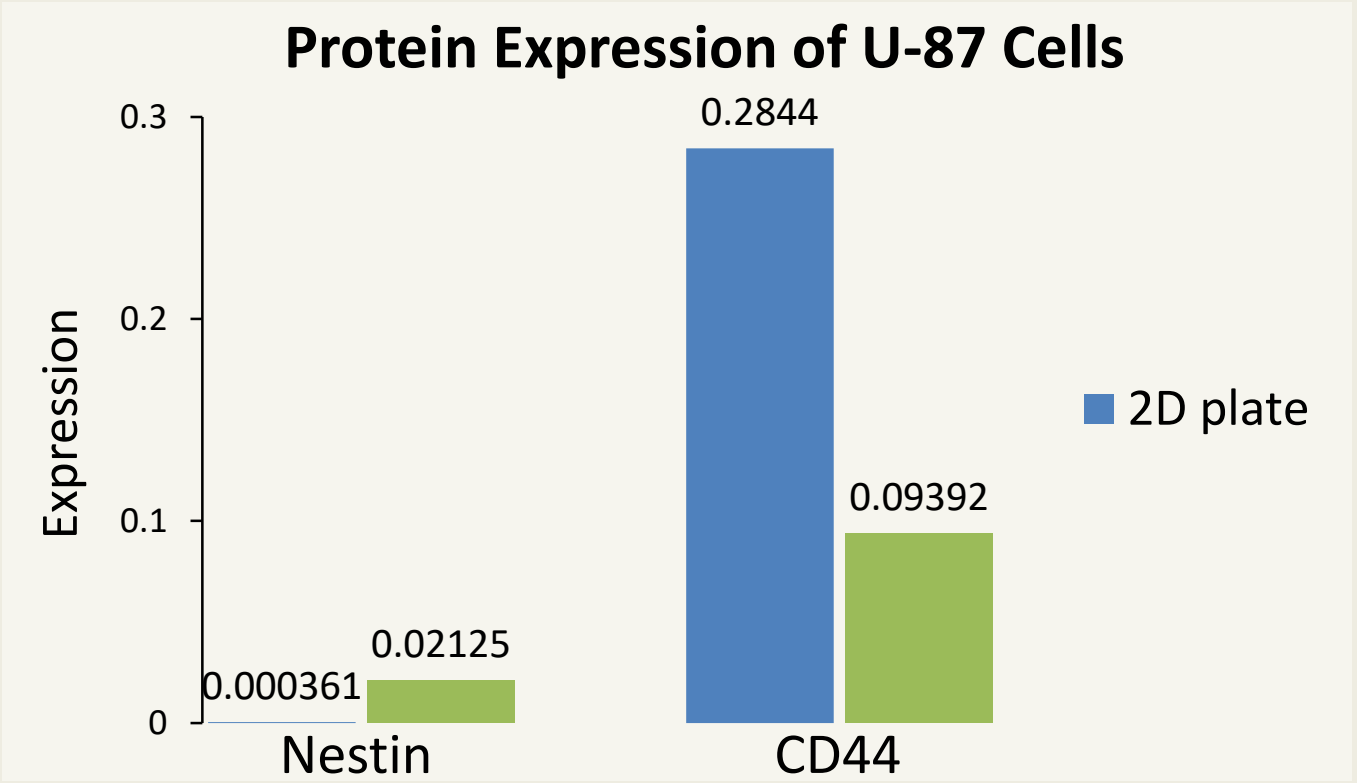


Diagram 2: *Illustration of detection in Western Blots.*

Results

- **Nestin** had increased expression in CHA scaffolds compared to 2D plates
- **CD44** had decreased expression in CHA scaffolds compared to 2D plates



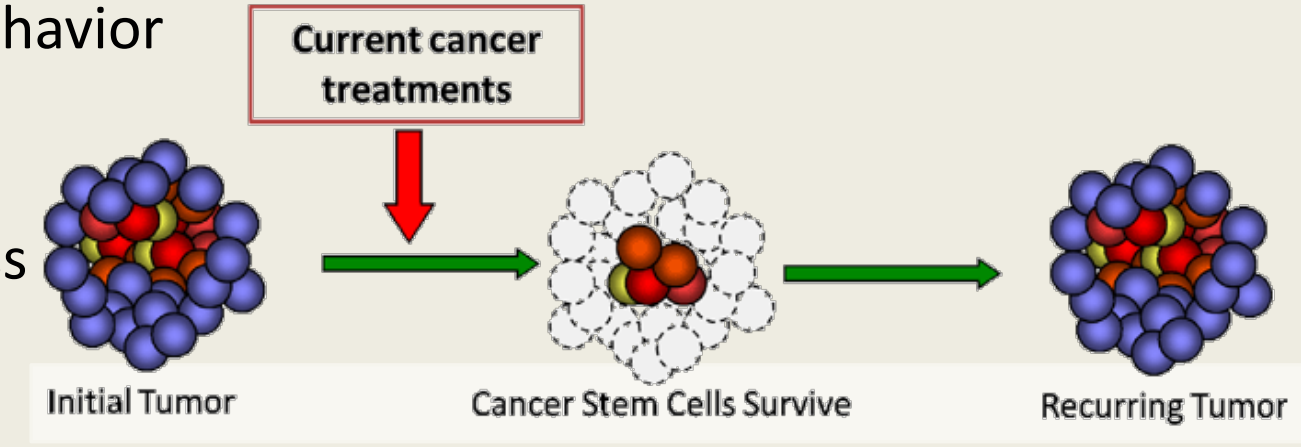


*Beta actin is a control protein that does not behave differently between 2D and 3D environments and can be used to calibrate other protein quantities.

Conclusion/Discussion

- CHA scaffolds support growth of tumor spheres, more similar to *in vivo* growth
- U-87 cells in scaffolds exhibit higher cancer stem cell characteristics as indicated by Nestin
- Unexpected decrease in CD44 expression possibly due to CD44 still attached to the hyaluronic acid in the scaffolds and was unable to be pulled out into lysate
- Follow-up tests needed to confirm cell behavior
- Use of 3D scaffolds could lead to
  - treatment with higher efficacy
  - less mice used and killed in the process
  - cheaper alternative for testing

Dr. Olivier Preynat-Seauve, 3R Research Foundation



Sources

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Thank You

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