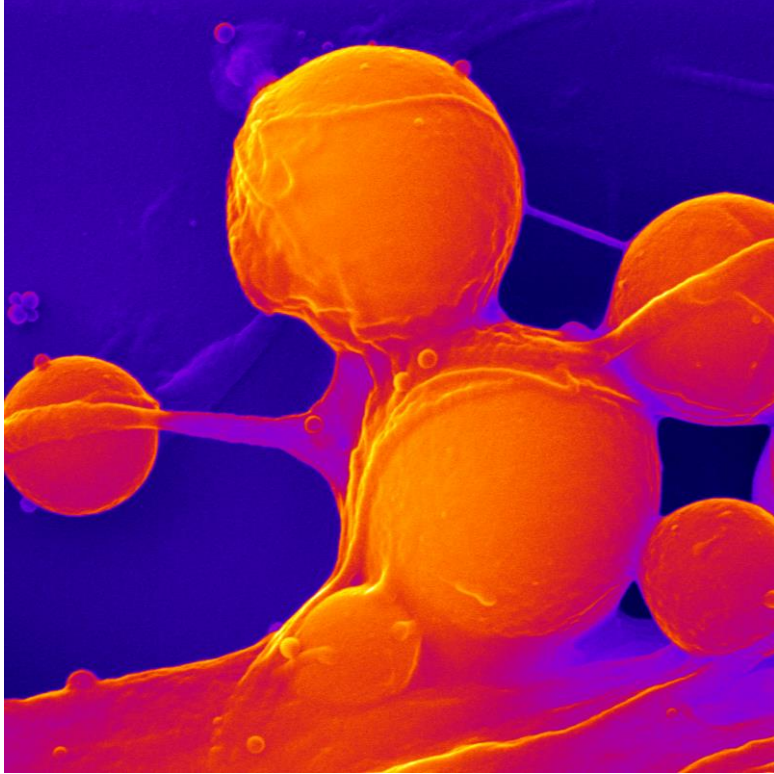




Biomanufacturing Architecture Programs Early Regenerative Signaling in Porcine Combat-Relevant Wounds

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Ronawk, Inc.¹

Disclosure

A.J. Mellott, Ph.D. – Owner, officer, and employee of Ronawk, Inc.

Challenges in Current MSC Derived EV Biomanufacturing

The industry currently relies on petri dishes, flasks, and bioreactors designed for microbial systems, not complex mammalian cells... this exhausts the cells.



...limitations contributing to

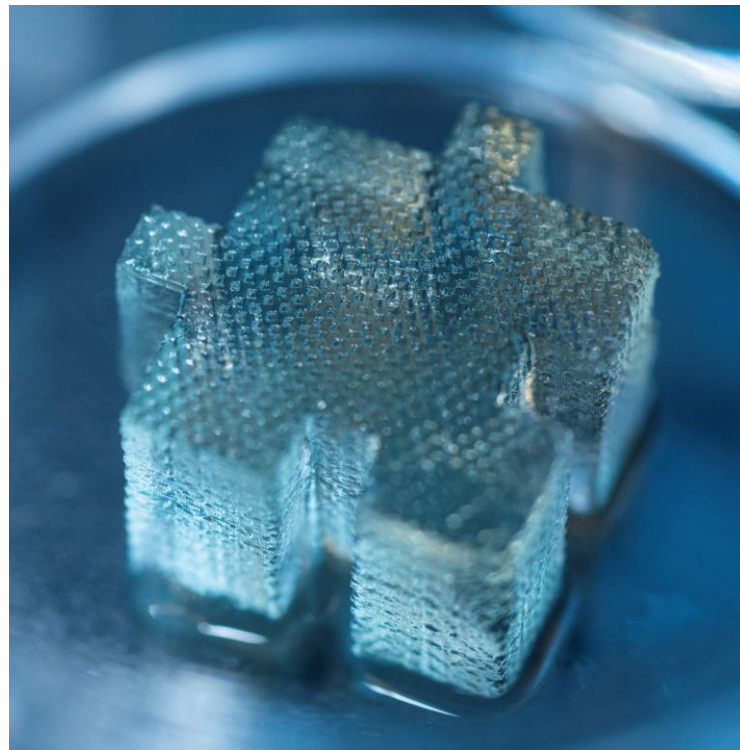
- **Senescence during expansion**
- **Phenotypic drift**
- **Inconsistent EV output**

Hypothesis

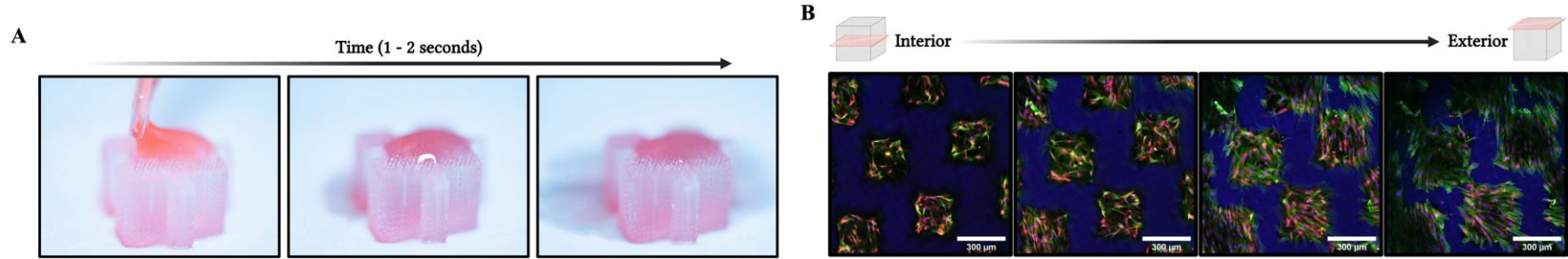
Upstream Architecture Influences Downstream Output

Architecture directly influences:

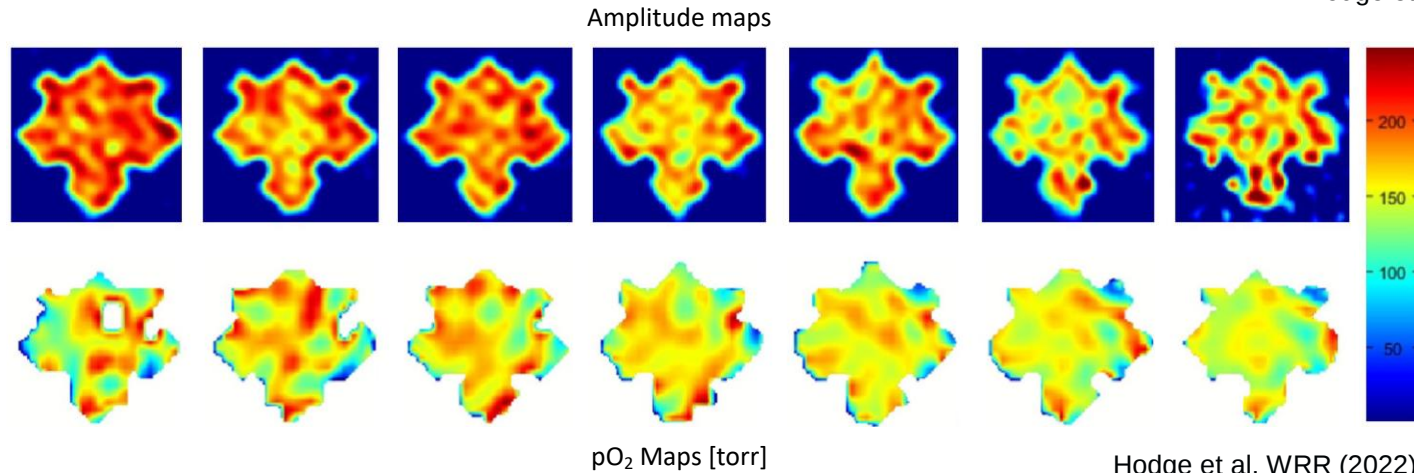
- MSC Phenotype
- Senescence
- EV Production
- EV Functional Potency



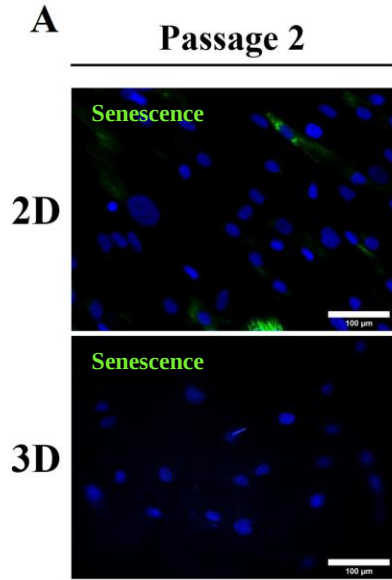
Porous Microarchitecture = Mass Transport of Nutrients, Cells and Gas



Hodge et al. WRR (2022)



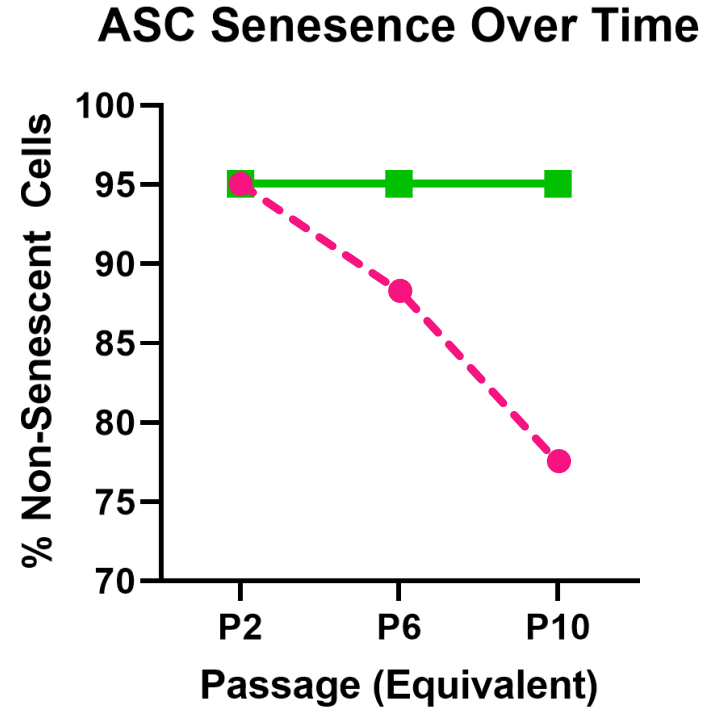
Delay of Senescence with 3D Tissue-Mimetic Culture



2D Culture → Passaged every 4-5 days

3D Culture → Passage-/Time-Equivalent

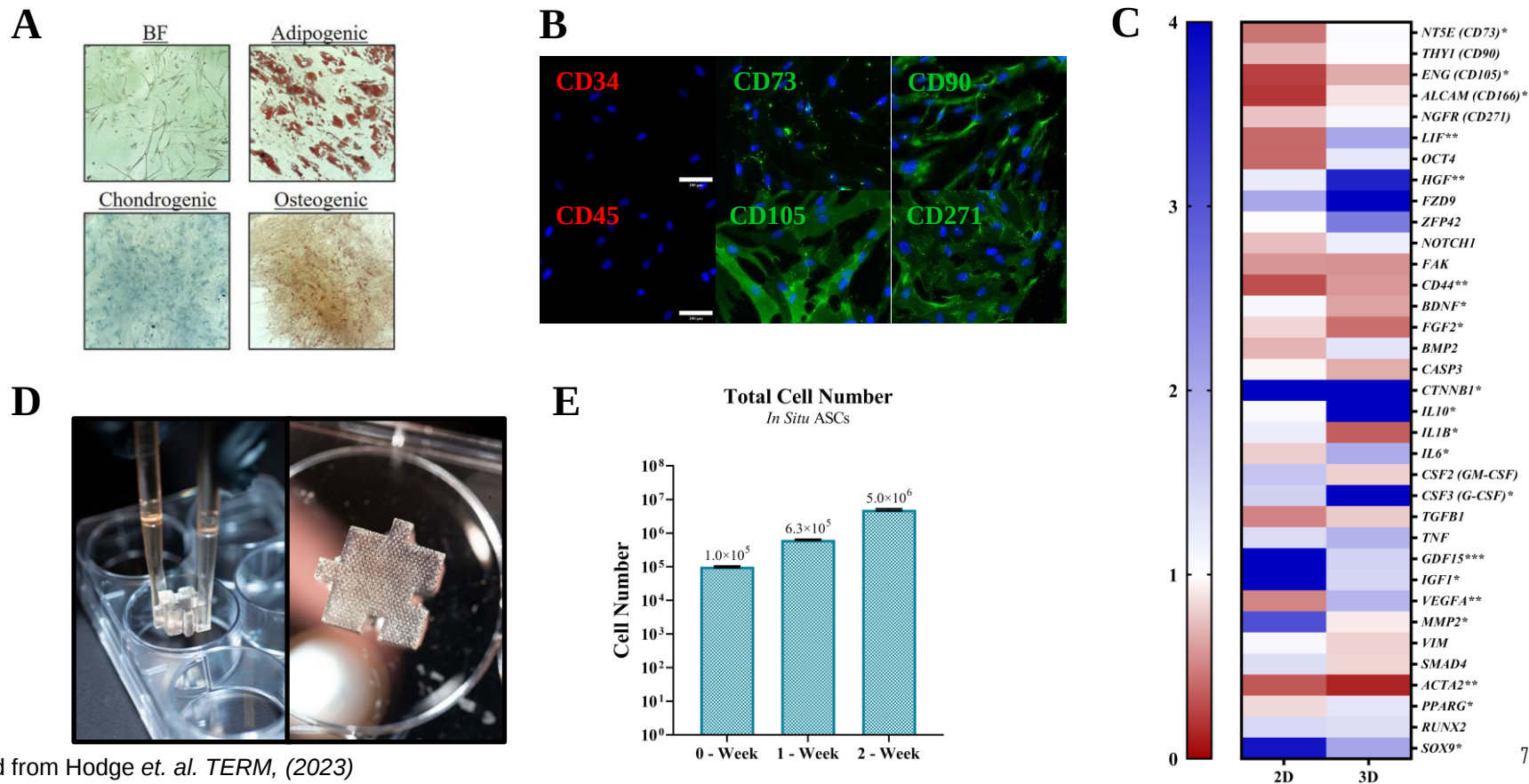
**P10 = 42 days of culture*



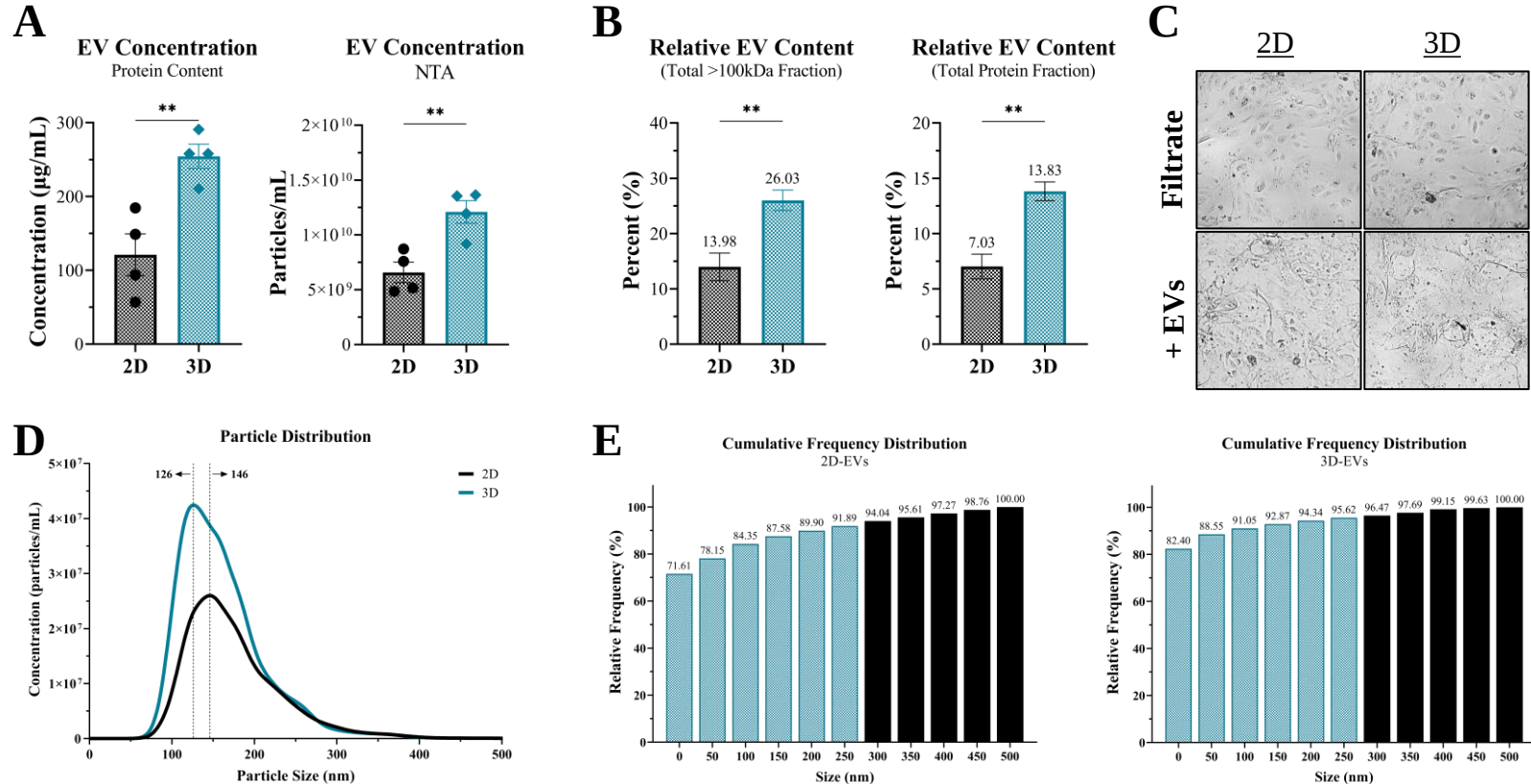
Adapted From Hodge et. al., (2023)

Tissue-mimetic culture of ASCs significantly limits progression of senescence over time

Retention of Stem-like Phenotype in 3D Tissue-Mimetic Culture

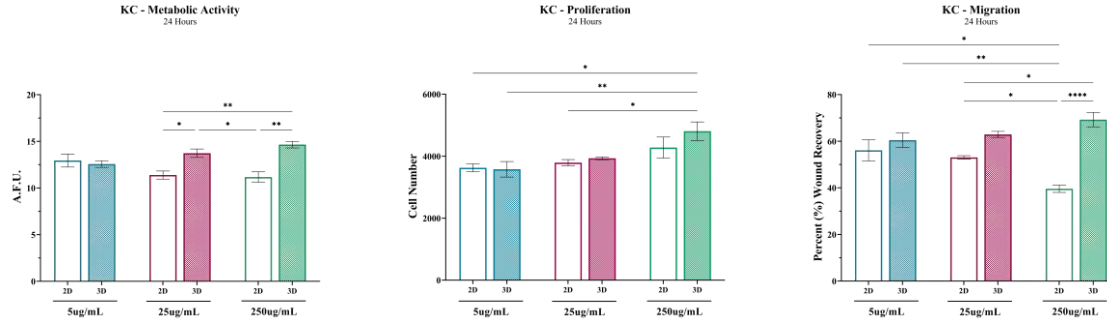


Tissue-Mimetic Culture Biases Secretion Toward EV Production

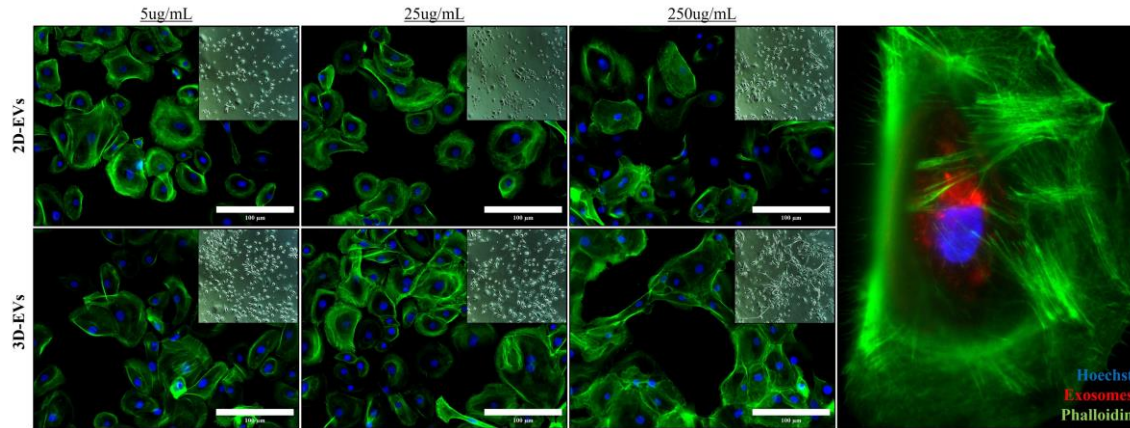


Uptake of EVs Derived from 3D Culture Influences Keratinocyte Activity

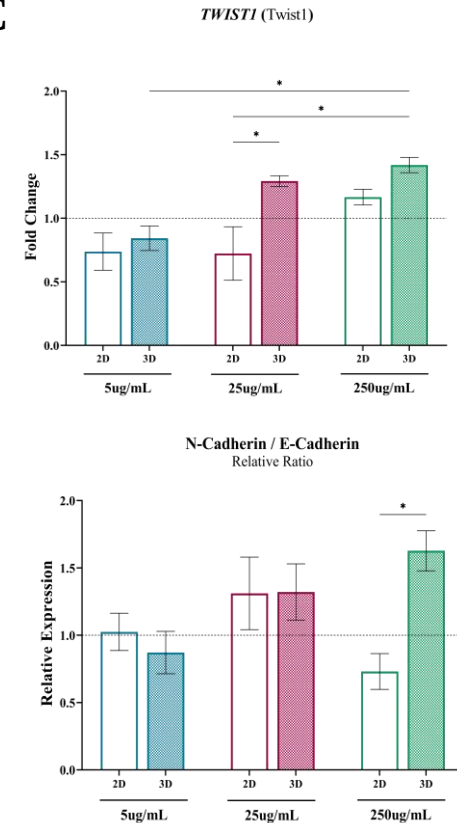
A



B

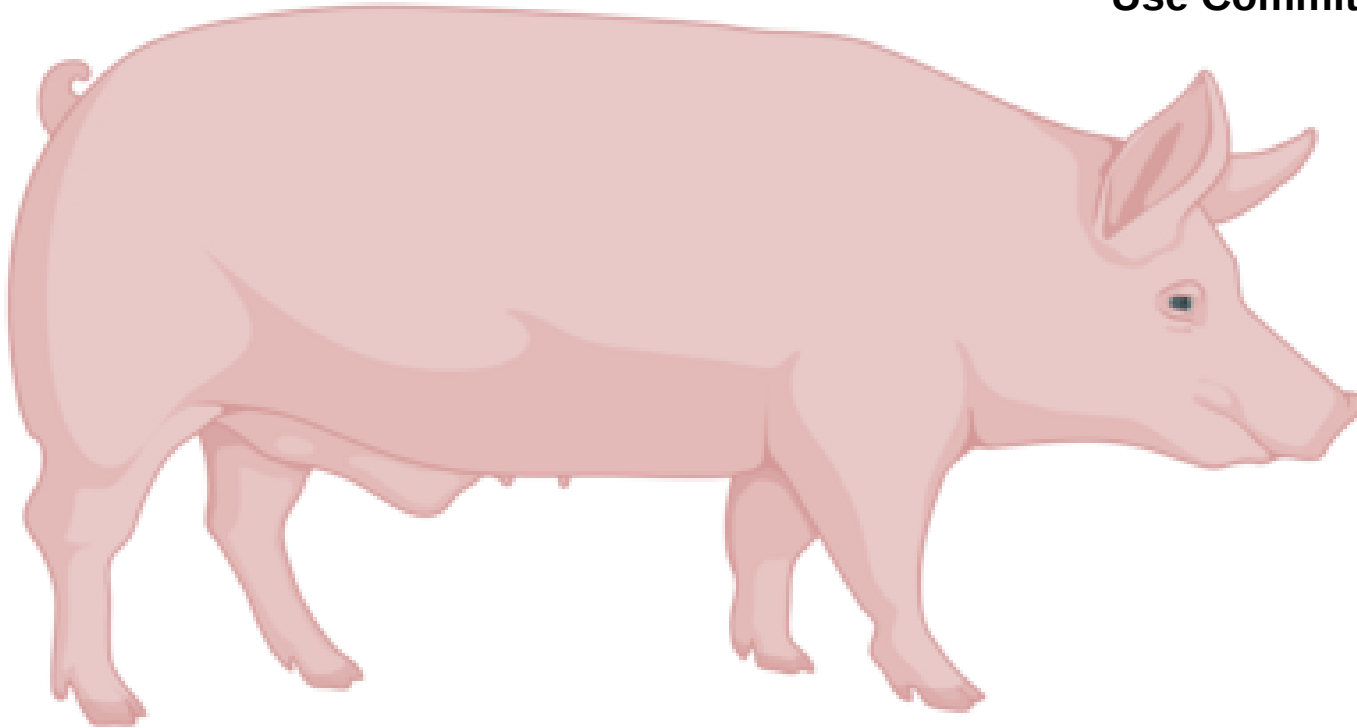


C

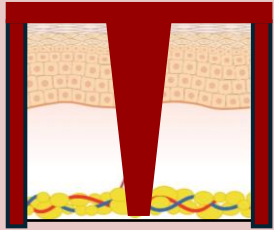


Animal Study

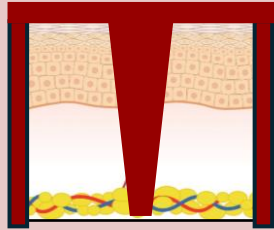
**All animal studies were conducted
under Institutional Animal Care and
Use Committee Approval**



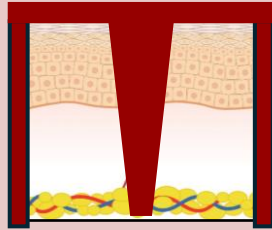
Animal Study



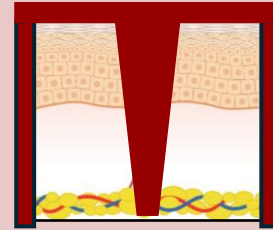
**0
Hour**



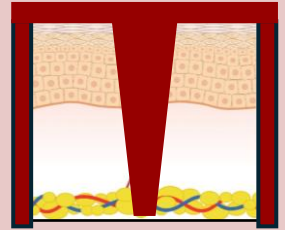
**4
Hour**



**8
Hour**

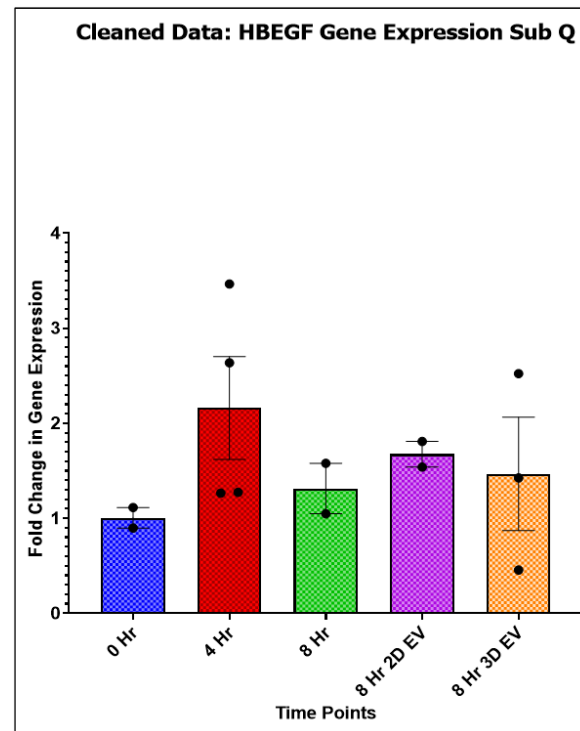
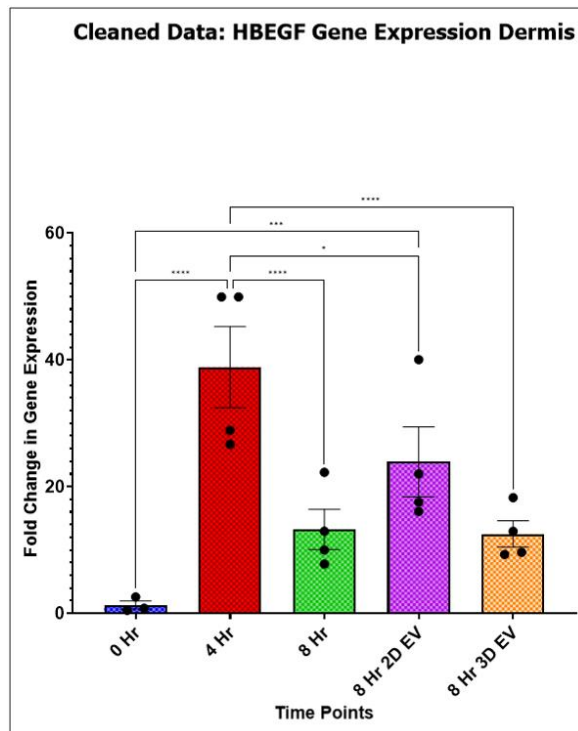
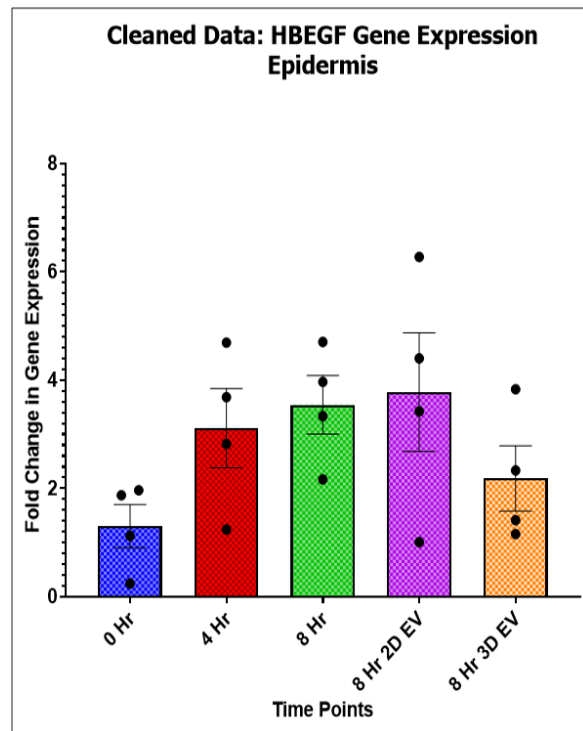


**8
Hour
2D EV**

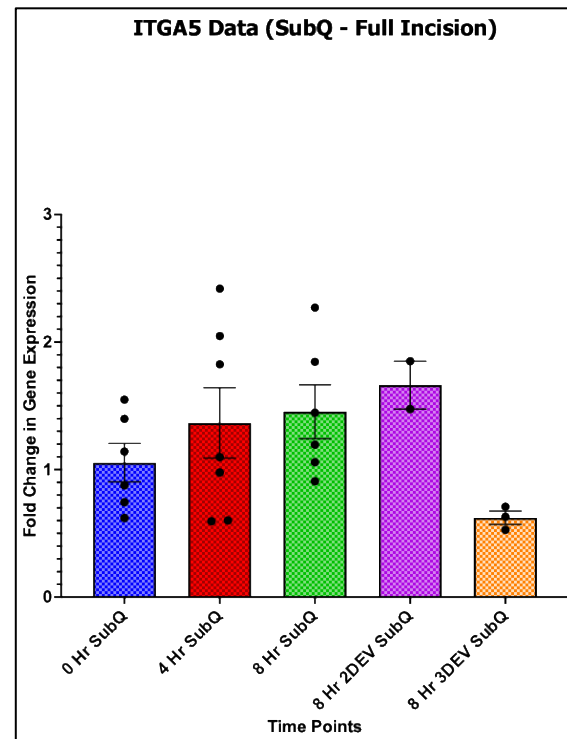
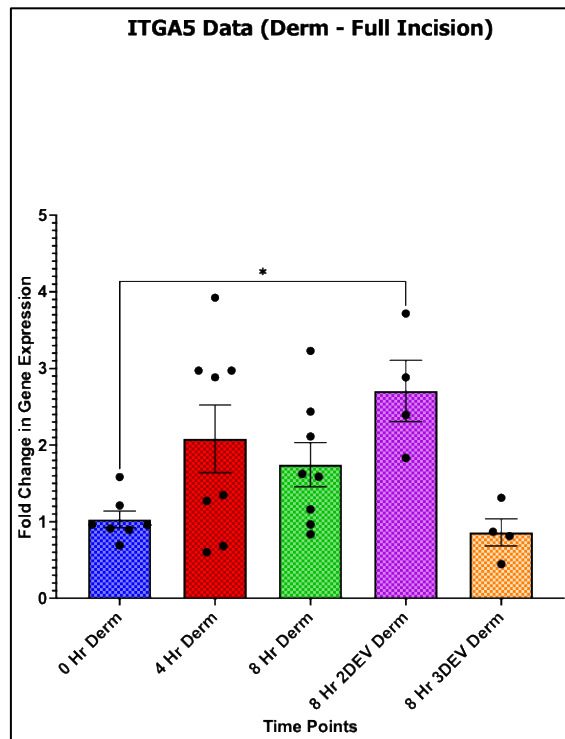
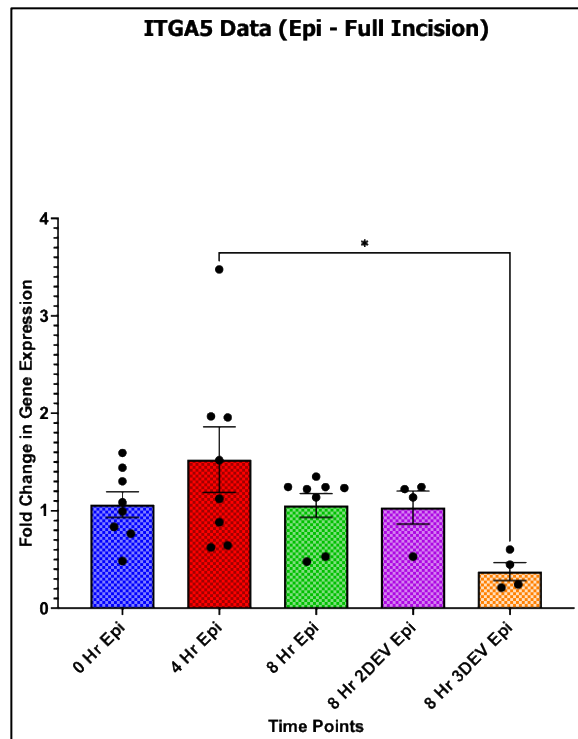


**8
Hour
3D EV**

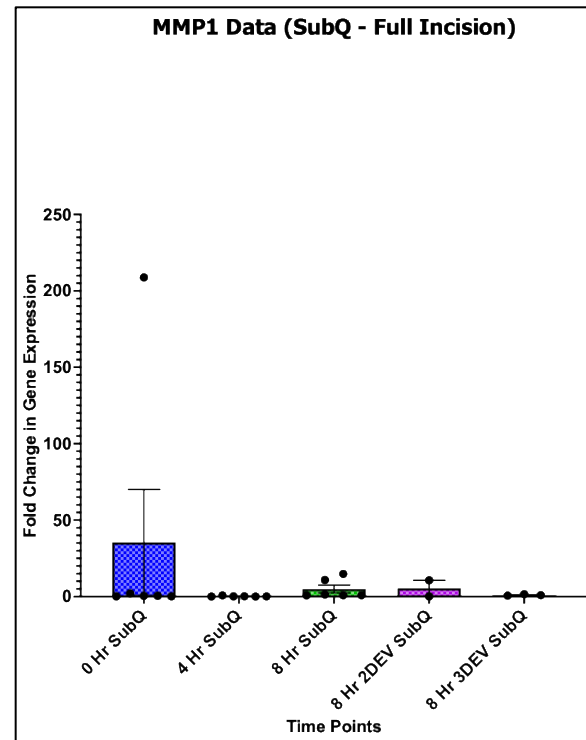
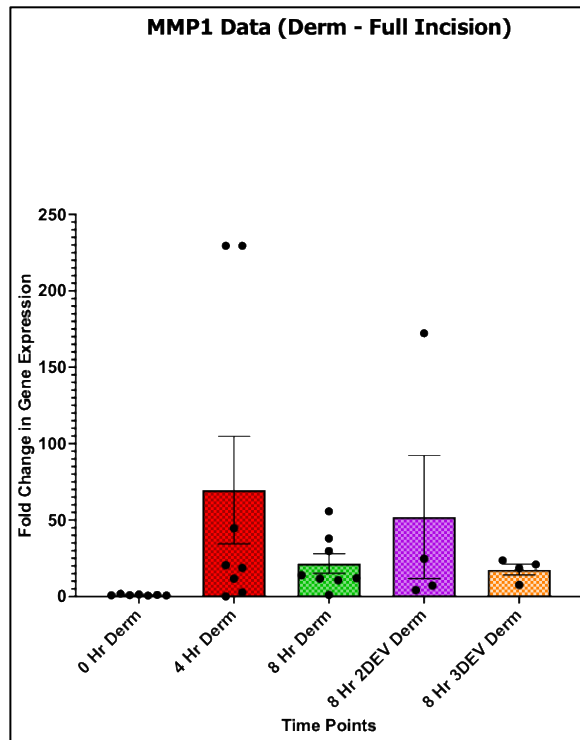
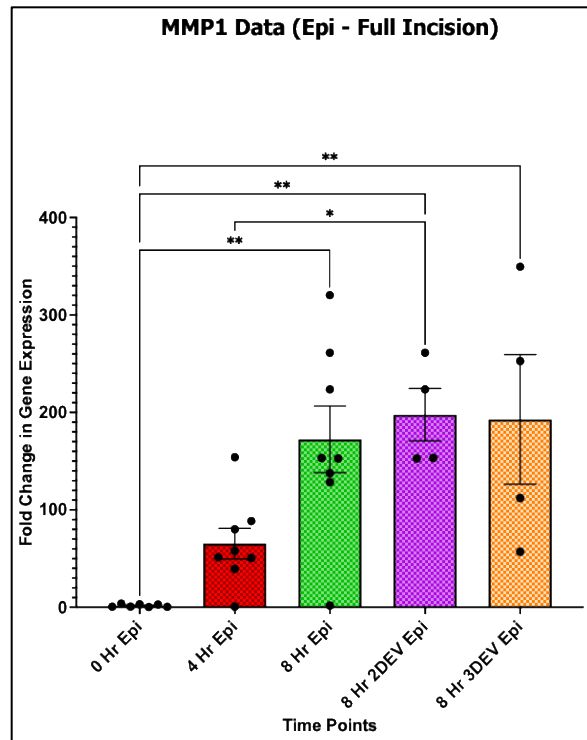
HBEGF



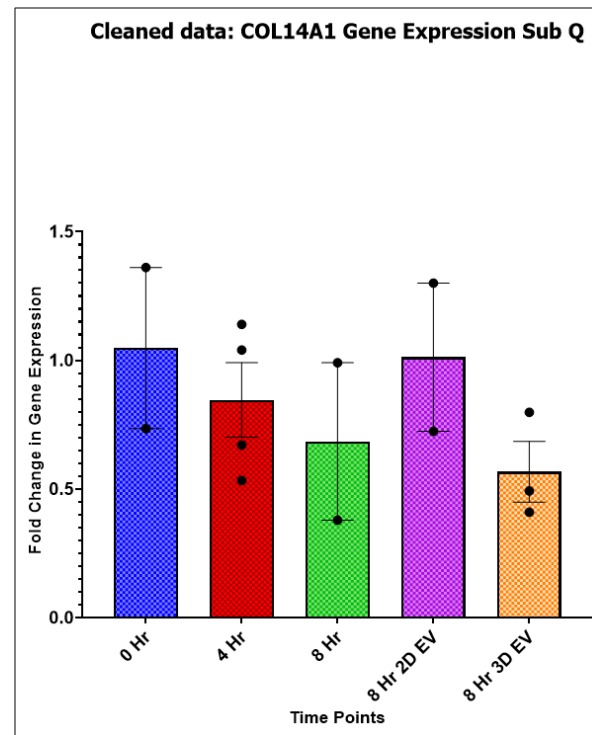
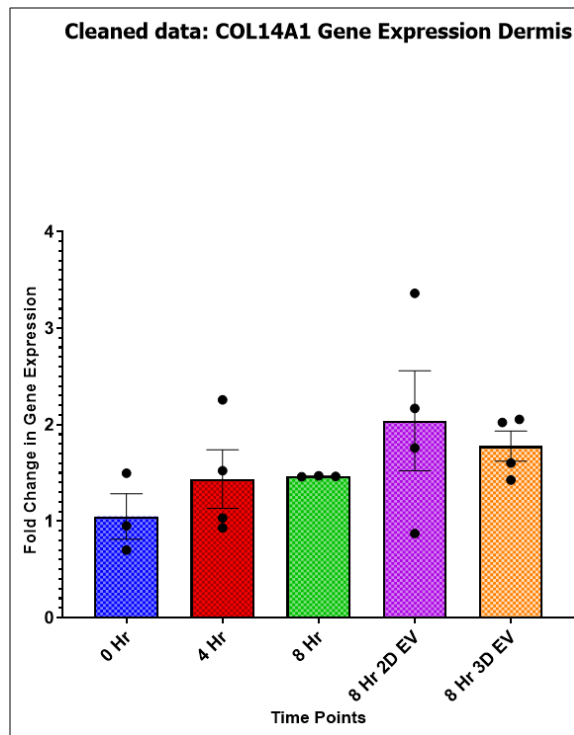
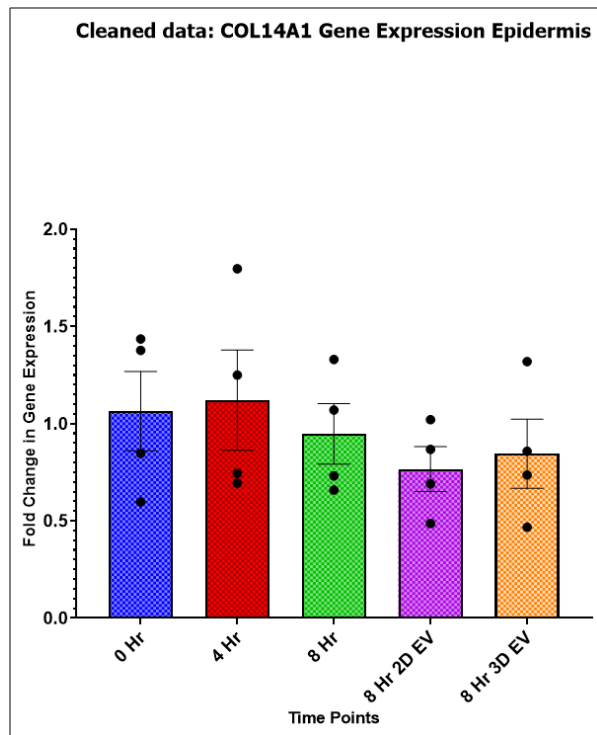
ITGA5



MMP1



COL14A1



Key Takeaways

- 1. Biomanufacturing architecture may influence the quality and potency of biologics**
- 2. MSC derived biologics, such as EVs, may be able to augment the body's own healing mechanisms**
- 3. The microenvironment plays an active role in how cells operate and interact**

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