Investigating the Response of Fibroblasts to Environmental Forces: An *in vitro* Platform to Study Migration and Wound Healing

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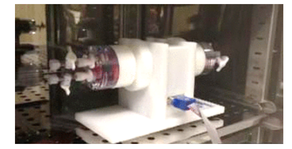
Introduction

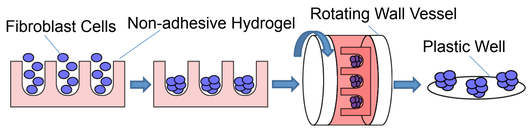
In order to effectively understand cellular processes, an *in vitro* model that closely models *in vivo* conditions must be first developed. Instead of studying cellular behavior in traditional two-dimensional (2D) culture, using a three-dimensional (3D) culture can provide an environment that more closely mimics *in vivo* conditions. Given the importance and wide applicability of 3D tissue culture, NASA scientists developed a bioreactor that presents simulated microgravity (SMG) and mimics the weightlessness experienced during space travel. Without the presence of gravity, cells in the bioreactor are allowed to maintain their 3D structure instead of flattening into a 2D monolayer, giving a more accurate representation of bodily conditions. Additionally, studies have shown the importance of substrate rigidity in mimicking cell behavior in the body. Using substrates of a stiffness more closely related to that found *in vivo* provides a more accurate depiction of cellular behavior as opposed to conventional culture surfaces such as glass.[1] Due to their critical role in wound healing,[2] this study focuses on the behavior of NIH 3T3fibroblast cells after being exposed to SMG in either 2D or 3D culture. Culture in 2D was also performed on polydimethylsiloxane (PDMS), a biocompatible material with tunable stiffness, before exposure to SMG to examine the effect of substrate rigidity.



NASA Developed Rotating Wall Vessel Bioreactor

I. Aggregates in Non-adhesive Hydrogels

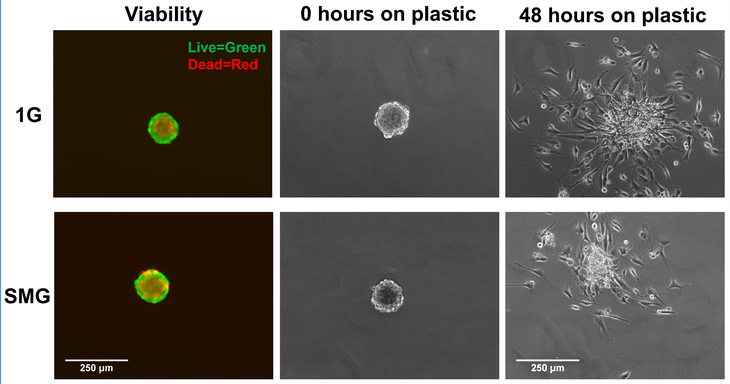
Experimental Design



- Fibroblast cells were seeded at 500 cells/ aggregate in agarose gels with microwells of diameter 400 µm and cultured for 24 hours
- Agarose gels were placed in rotating wall vessel at 20 rpm for SMG or kept in 1G for control for 24 hours
- Fibroblast aggregates were transferred from agarose gels to plastic 12-well plates
- Live/dead analysis of aggregates was performed immediately upon removal from rotating wall vessel (48 hours after initial aggregate formation)
- Cell migration from fibroblast aggregates was observed over 48 hours

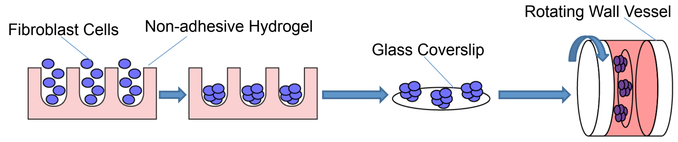
Results:

- SMG fibroblasts aggregates contained more dead cells around edges
- SMG fibroblasts were less migratory than 1G fibroblasts



II. Aggregates on Glass Coverslips

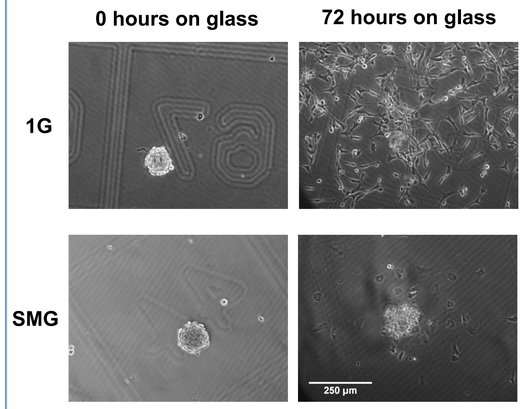
Experimental Design



- Fibroblast cells were seeded at 500 cells/aggregate in agarose gels with microwells of diameter 400 µm and cultured for 24 hours
- Fibroblast aggregates were transferred from agarose gels to gridded glass coverslips coated with fibronectin
- Glass coverslips were placed in rotating wall vessel at 20 rpm for SMG or kept in 1G for control for 24 hours
- Cell migration from fibroblast aggregates was then observed over 48 hours

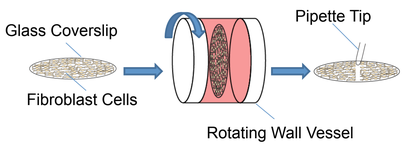
Results:

- SMG fibroblasts were less migratory than 1G fibroblasts



III. Monolayer on Glass Coverslips

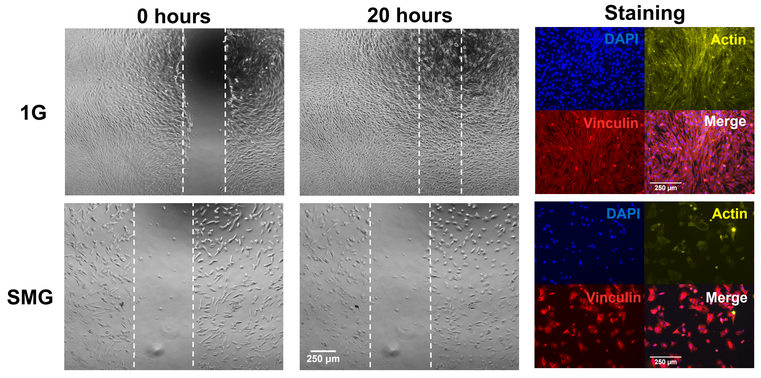
Experimental Design



- Fibroblast cells were seeded at a density of 26,000 cells/cm^2 onto 18 mm glass coverslips coated with fibronectin
- After cells attached for 24 hours, the coverslips were placed in rotating wall vessel at 20 rpm for SMG or kept in 1G for control for 24 hours
- Upon removal from SMG, or after 24 hours for 1G, coverslips were scratched with a pipette tip and migration was analyzed with time lapse microscopy (20 hours)
- Cells were stained for vinculin and actin and 4',6-diamidino-2-phenylindole (DAPI) was used to visualize cell nuclei

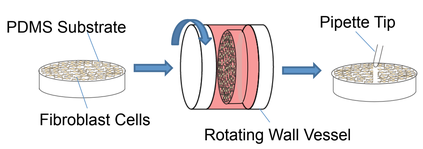
Results:

- SMG fibroblasts were unable to close scratch within 20 hours
- SMG fibroblasts were more rounded and less extended than 1G fibroblasts
- Vinculin staining showed localized focal adhesions in SMG fibroblasts and homogenous focal adhesions in 1G fibroblasts
- Actin staining suggested alterations in the actin stress fibers of SMG fibroblasts



IV. Monolayer on PDMS Substrate

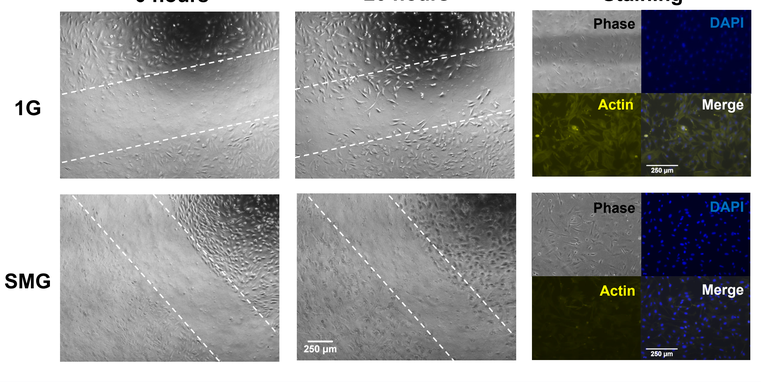
Experimental Design



- Fibroblast cells were seeded at a density of 26,000 cells/cm^2 onto PDMS of stiffness 5 kPa —1.72 MPa coated with fibronectin
- After cells attached for 24 hours, the PDMS was placed in rotating wall vessel at 20 rpm for SMG or kept in 1G for control for 24 hours
- Upon removal from SMG, or after 24 hours for 1G, the PDMS was scratched with a pipette tip and migration was analyzed with time lapse microscopy (20 hours)
- Cells were stained for actin and DAPI

Results:

- SMG fibroblasts were unable to close scratch within 20 hours
- SMG fibroblasts were more rounded and less extended than 1G fibroblasts
- Actin staining suggested alterations in the actin stress fibers of SMG fibroblasts



Conclusions and Future Directions

Overall, fibroblasts exposed to SMG showed changes in viability, migration, and intracellular organization. SMG also affected cell morphology, resulting in a more rounded and less extended cellular structure. Future studies may investigate the effect of conditioned fibroblast media from SMG on 2D fibroblast wound healing or the effect of conditioned media from 1G fibroblasts on 2D SMG fibroblast wound healing.

Acknowledgements

This project was generously funded by the Karen T. Romer UTRA, the Leadership Alliance, NSF REU, and the NASA RI Space Grant. Thank you to Molly Boutin, Yu-Ting Dingle, and Liane Livi for their invaluable insight and discussion.

References

(1) Guo, Wei-hui, et al. (2006). “Substrate Rigidity Regulates the Formation and Maintenance of Tissues.” Biophysics Journal. 90, 2213-220. 10.1529/biophysj.105.070144. (2) Liang, C.-C., A. Y. Park, et al. (2007). "In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro." Nat. Protocols 2(2): 329-333. (3) Vunjak-Novakovic, Gordana, et al. (2002). “Microgravity Studies of Cells and Tissues.” Ann. N.Y. Acad. Sci. 974: 504–517.