Effect of Simulated Spaceflight on Wound Healing Responses

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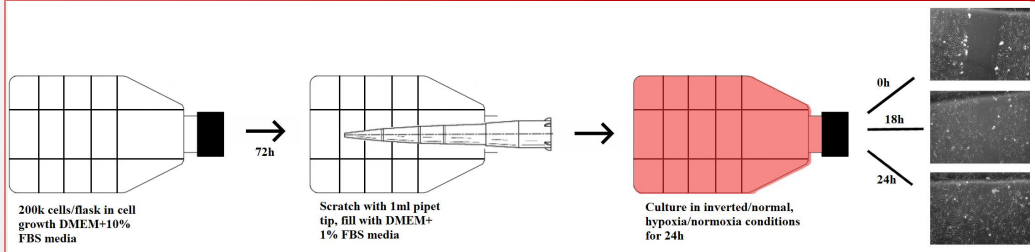
Abstract

Amidst growing interests in human space exploration and private spaceflight commercialization, it is ever important to understand the effects of spaceflight on the human body. The purpose of this study is to investigate the effects of simulated microgravity in conjunction with hypoxia on wound healing responses, both hypothesized to be hindered by spaceflight conditions. Wound healing was observed in vitro through migration assay experiments under hypoxic conditions and inverted tissue culture flask configuration as a model to simulate microgravity (µg). Results suggest that fibroblast migration is not inhibited by inverted flask model over the 24-hour study period, and that hypoxia in fact enhances migration in this cell type. Future experiments with improved methodology for longer observation of wound closure are necessary to better determine the effects of simulated microgravity using this model.

Background

Several in vitro studies have found that cell migration, a major mechanism of wound healing, is significantly hindered under µg conditions. Migration assays performed on fibroblasts found that cell migration was significantly impaired when subjected to simulated microgravity by Rotary Cell Culture System (RCCS) or Random Positioning Machine (RPM)[1][2]. There are few studies which explore secondary factors that may affect wound healing responses. Spaceflight hypoxia has long been a concern for astronauts, and can impair wound healing due to insufficient oxygen delivery to the damaged tissue[3]. NASA has determined that future EVA missions will be under the Exploration Atmosphere of 8.2 psia and 34% O2 with potential prebreathe gas mixtures of less than 100% O2, resulting in mild hypobaric hypoxia[4]. In this study, we investigate the effects of simulated microgravity (µg) and hypoxia on fibroblast cell migration responses. We devised a simple, low-resource model for simulating µg by flipping (referred onwards as inverted configuration) the media-filled tissue culture flask such that adherent cells were upside down. We seek to evaluate this model for use in studying wound healing in vitro in simulated spaceflight conditions.

Materials and Methods



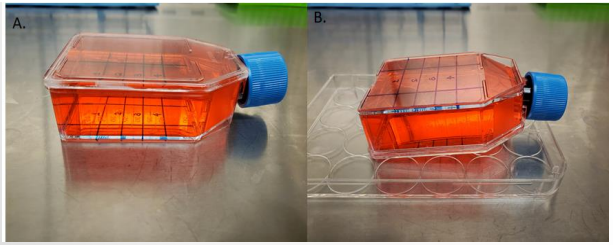


Figure 1. (A) 200,000 fibroblast cells were seeded in a T25 flask and left to culture for 48-72 hours in 5ml growth media DMEM+10% FBS per flask until 90-100% confluent. The bottom of each flask was marked with a 2x4 grid to indicate the center. Upon reaching 90-100% confluency, wounds were induced by inserting a sterile 1ml pipet tip into the T25 flask to remove cells down the center. Each flask was fully filled with DMEM +1% FBS and placed into respective hypoxia/normoxia and µg/1g conditions. Conditions were 37C, 5% CO2, 1% O2 or 37C, 5% CO2, 20% O2 for hypoxia and normoxia respectively. Additionally, flasks were incubated either upright, standard configuration, or were flipped upside down ("inverted") to simulate µg. Images were taken at 0, 18, and 24 hours over a 24 hour study, providing measurable wound area. **(B)** Cells were cultured in standard normal configuration, underwent scratch, and was filled with media and flipped upside down to simulate µg.

Results

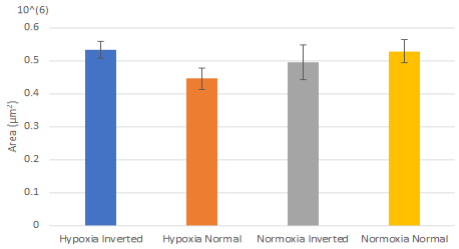
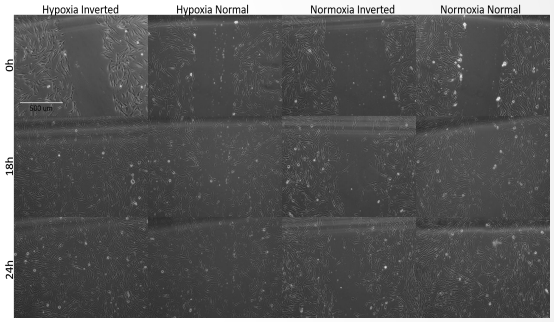


Figure 3. Initial wound area (µm2) at 0 hours. Wounds were made by inserting a sterile 1ml pipet tip into the opening of a T25 flask, removing cells down the middle of the flask. Scratches were performed by hand, resulting in the initial wound areas displayed. Images were taken immediately after inducing scratch in fibroblast cells, and image analysis was performed to record starting wound area for each condition. Wounds that were too thin, or wound areas too small, were more likely to close prior to seeing the full effect of the modeled *µg*.

Figure 4. One scratch was made per flask, producing 4 samples to be imaged according to marked 2x4 grid. After inducing wounds, cells were incubated in respective configurations and conditions over the course of a 24 hour study. Cells were imaged at 0, 18, and 24 hours to observe cell migration and wound closure responses. Images produced four wound areas per flask at 0, 18 and 24 hours. Area between wound boundaries is measured from images to produce \% wound closure.
Percent wound closure was calculated as follows:
[(wound area at t0h- wound area at t24h)/(wound area at t0h)]*100\%



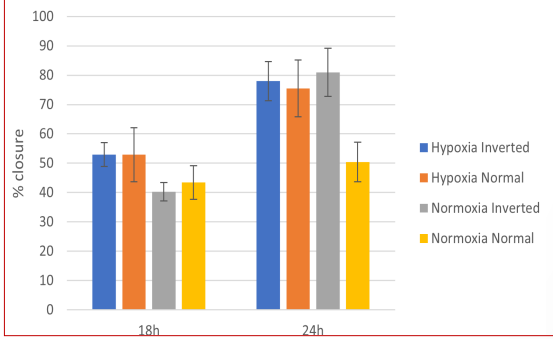


Figure 5. Percent (%) wound closure for each condition was calculated and averaged across samples. % wound closure was considered to be 0% at the initial timepoint, 0 hours, and % closure was calculated at 18 and 24 hours relative to this initial wound area At 18 hours, cells in hypoxia exhibit greater wound closure than cells in normoxia. By 24 hours, normoxia inverted cells exhibit slightly higher wound closure. Both hypoxia groups show greater wound closure than normoxia normal group at both timepoints. Hypoxia inverted cells exhibited 77.95% average % closure and hypoxia normal cells exhibited 75.44%. Data is presented as mean±SEM (n=8-12).

Conclusion

Results showed that hypoxia enhanced wound closure, and that the modeled microgravity did not inhibit migration. Future experiments using this model will require adjustments in methodology that allow for longer observation of fibroblast migration responses in hypoxia and simulated microgravity.

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Acknowledgments

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