

## Gravitational and Space Research

# Impact of Simulated Microgravity Environment on Bioprinted Tissue Constructs

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### Abstract

*Microgravity significantly impacts cellular functions and physiological systems, leading to oxidative stress and cellular dysfunction. This study investigated the effects of simulated microgravity on bioprinted vascular tissue constructs made with human skin fibroblast cells encapsulated in GelMA-based bioink. The bioprinting parameters were optimized to obtain tissue constructs with high structural integrity and cellular viability. Cellular viability remained high despite some cell death, likely caused by shear stress during bioprinting or limited nutrient availability while maintaining the samples in a bioreactor. The samples were stained with DHE solution to characterize the oxidative stress and imaged using a confocal microscope. The study revealed that oxidative stress, quantified by reactive oxygen species (ROS) levels, increased significantly after 48 h of simulated microgravity exposure. The ROS levels rose from 24 h to 48 h and then there was a subsequent decrease at 72 h, which reflects typical cellular responses to stress from microgravity such as cell adaptation and possible recovery mechanisms. These findings highlight the need for targeted strategies, such as incorporating antioxidants or mitochondria supporting compounds into the bioinks and dynamic culture systems to mitigate the effects of microgravity-induced oxidative stress.*

### Keywords

microgravity • bioprinted vascular tissue • cellular viability • oxidative stress

## Introduction

Microgravity is one of the most notable stressors to living organisms that spaceflight might entail, mediating direct effects on cellular functions and physiological systems. *In vitro* experiments demonstrated that the microgravity environment produces cytoskeletal rearrangements and alters proliferation and apoptosis related to impaired cellular communication or signaling pathways (Bizzarri et al., 2015; Huang et al., 2018; Corydon et al., 2023). The physiologic effects include muscle atrophy, bone resorption, cardiovascular deconditioning, and immune responsiveness. The knowledge gained through investigating these effects helps formulate countermeasures for astronauts and refine practices on Earth (Nguyen et al., 2021; Sharma et al., 2023; Beckett et al., 2024).

In microgravity, increased reactive oxygen species (ROS) production and mitochondrial morphology changes lead to increased oxidative stress and mitochondrial dysfunction, causing oxidative damage to multiple organ systems, including cardiovascular and immune systems (Hunt et al., 2024; Neje et al., 2024; Rudolf & Hood, 2024). Oxidative stress also hampers wound healing and endothelial function and accelerates cellular senescence, posing challenges for long-term space missions. Antioxidant therapies and mitochondrial-targeted approaches are crucial for mitigating

these effects and addressing Earth-related oxidative stress-induced diseases (Ferranti et al., 2020; Varesi et al., 2022; Lei et al., 2024).

Opportunities to study the effects of gravity variations through space flights are limited due to high costs and the inability to repeat experiments consistently. Therefore, microgravity simulators have been developed to study cellular-level effects similar to those experienced during or after space travel (Atrooz et al., 2021; Miglietta et al., 2023).

Results obtained in our study using random position machines (RPMs) promote congruency between the measured effects of cellular response to that experienced under spaceflight conditions. These effects include cytoskeletal disorganization, oxidative stress, and changes in mitochondrial activity and gene expression, which can impair cell structure, energy production, and long-term viability (Nguyen et al., 2021). However, RPMs have some limitations; for example, the gravity vector averaging to zero does not eliminate the presence of gravity completely, thereby potentially underestimating the effects of true microgravity encountered in space (Kim et al., 2017). RPMs also introduce artifacts such as shear stress and fluid motion. These forces are important, as they can independently influence mechanosensitive signaling pathways, oxidative stress

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responses, and cytoskeletal remodeling, making it challenging to separate microgravity-induced effects from motion-related artifacts (Kouznetsov, 2022; Cortés-Sánchez et al., 2023). Despite some of these limitations, RPMs are highly valuable and cost-effective tools to augment space biology research (Wuest et al., 2015; Graf et al., 2024).

Animal models like hindlimb unweighting (HU) face limitations due to species differences, stress responses, and experimental constraints. These issues underscore the importance of refining models to improve their applicability to human studies (Thippabhotla et al., 2019).

Two-dimensional (2D) cell monolayers have been shown to inadequately represent the physiology of *in vivo* three-dimensional (3D) tissues or organs, primarily due to the significantly different mechanical and biochemical properties present in tissue architecture (Murphy and Atala, 2014).

3D bioprinted tissue constructs will be helpful in modeling tissue function and will act as pharmacological targets to manage diseases, owing to their ability to more closely mimic *in vivo* tissues and the surrounding extracellular matrix (ECM). Developments in understanding the many roles that the ECM plays as a key regulator of tissue architecture and cell–cell communication further highlight the need for 3D tissue culture models for analyzing diseases. However, enhancement of the function of bioprinted tissues is necessary to generate structures that mimic the intricate architecture and complexity of native tissues. 3D cell culture models are anticipated to provide significant advantages over their 2D counterparts, including more physiologically relevant representations of cell morphology, proliferation gradients, response to drugs, gene expression, and overall cell behavior. Manually producing these 3D culture models has proven difficult and expensive for many researchers, often resulting in high scaffold-to-scaffold variability. 3D bioprinting technology has emerged as a viable solution to this problem, offering increased efficiency and reproducibility (Cukierman et al., 2001; Pampaloni et al., 2007; Groll et al., 2016; Hinderer et al., 2016;; Xie et al., 2020; Milojević et al., 2023).

In 3D bioprinting, bioink is the principal material that supports the encapsulation and deposition of living cells to create complex structures for tissue engineering. It enables the creation of precise, scalable, and functional tissue constructs by mixing bioink and cells. Bioinks must mimic native ECM properties, maintain elasticity, and ensure smooth extrusion during printing. Extrusion-based bioprinting is particularly versatile but requires precise control of factors like bioink viscosity, nozzle diameter, and printing speed to ensure structural stability and cell viability. Advancements in bioprinting offer scalable and reproducible solutions for creating tissue constructs, facilitating research into disease modeling and regenerative medicine (Snezhkina et al., 2019; Afzal et al., 2023; Tripathi et al., 2023, 2025).

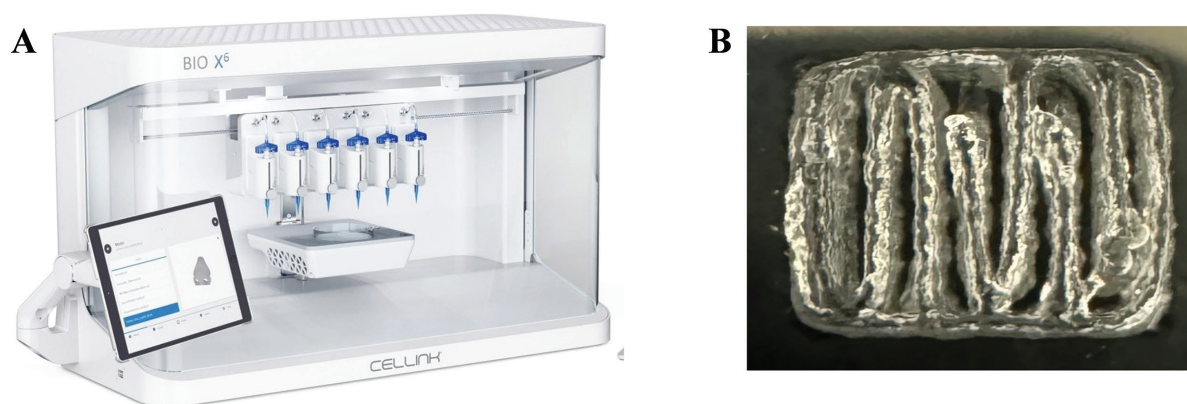
The objective of this paper is to analyze the effects of simulated microgravity as obtained using an RPM on bioprinted vascular tissue constructs. The oxidative stress encountered in a simulated microgravity environment is characterized by analyzing the ROS levels. This study will contribute insights into the cellular adaptation mechanisms and oxidative damage under microgravity that impact the astronaut's health and treatments for oxidative stress-related diseases on Earth.

## Materials and Methods

The human skin fibroblast cells strain of CRL 2522 was purchased from ATCC. Fibroblast cells were cultured using growth media consisting of 85% Eagle's minimum essential medium (EMEM), 10% fetal bovine serum (FBS), and 5% Gibco™ penicillin-streptomycin (10,000 µl/ml) purchased from Fisher Scientific. For cell culture, cells were thawed and centrifuged, resuspended in media, and transferred to T-75 cm<sup>2</sup> flasks for cell growth in the incubator at 37°C and 5% CO<sub>2</sub>. The cell density achieved ranged from 1 x 10<sup>5</sup> cells/ml to 3 x 10<sup>6</sup> cells/ml for mixing with bioink.

The bioink used in this study was formulated using gelatin methacrylate (GelMA) powder obtained from Advanced Biomatrix. The GelMA powder was dissolved at a concentration of 10% (w/v) in reconstitution agent P, a buffer designed to maintain physiological conditions for cell culture applications. To enable photo-crosslinking, lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was added at a concentration of 0.25% (w/v). A viscosity flow sweep confirmed the appropriate shear-thinning behavior of the prepared bioink and identified the optimal print temperature of 23.5°C. Further analysis of viscoelastic properties (storage modulus and loss modulus) confirmed the desired consistency of the GelMA bioink. Subsequently, the prepared bioink was mixed with CRL 2522 cells suspended in culture media at a 10:1 (v/v) ratio to produce the final cell-laden bioink. This was achieved by loading the bioink and the cell suspension into separate syringes connected via a Luer lock. The two components were combined by carefully passing them back and forth between the syringes until a homogeneous mixture was achieved. Each milliliter of the bioink yielded approximately 2–4 bioprinted samples.

An extrusion-based bioprinter (Cellink BioX6) shown in Fig. 1A was used for bioprinting vascularized tissue constructs. Fig. 1B illustrates vascular tissue constructs with a rectilinear grid pattern featuring channels designed explicitly for perfusion. Table 1 outlines critical parameters used for bioprinting of tissue constructs with optimal cell viability and structural integrity. Parameters like cell density, needle diameter, and print speed primarily influence the print resolution and cellular distribution, while temperature, pressure, and layer height



**Figure 1.** (A) Cellink BioX6 extrusion-based bioprinter; (B) Bioprinted vascular constructs.

**Table 1.** Bioprinting parameters.

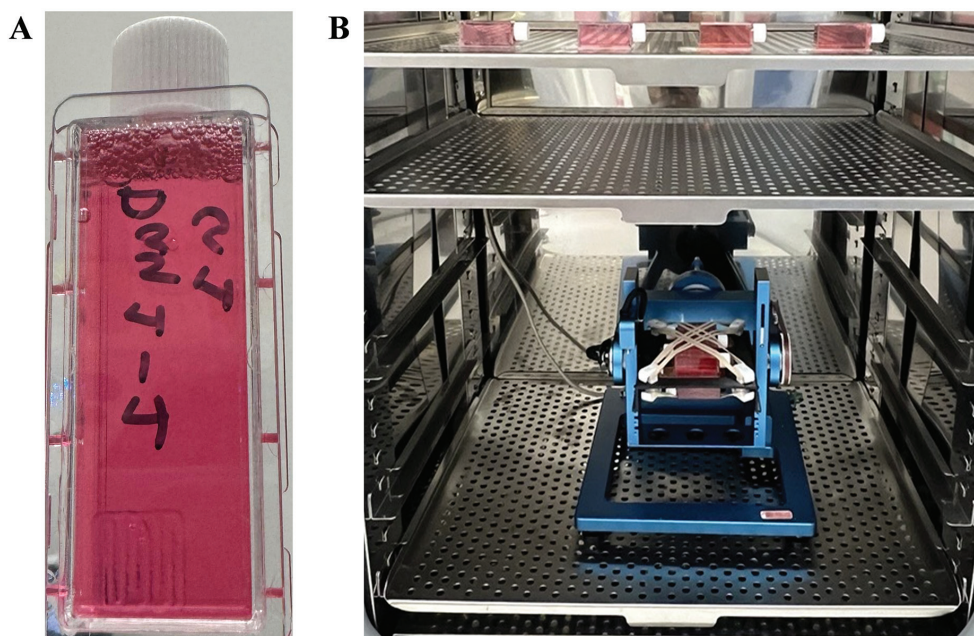
| Parameter                                  | Range of Values                                    |
|--------------------------------------------|----------------------------------------------------|
| Cell density                               | $1 \times 10^5$ cells/ml– $3 \times 10^6$ cells/ml |
| Nozzle diameter                            | 22 gauge                                           |
| Print speed                                | 7 mm/s                                             |
| Printhead temperature                      | 23.5°C                                             |
| Print bed temperature                      | 15°C                                               |
| Layer height                               | 0.41 mm                                            |
| Pressure                                   | 40–75 kPa                                          |
| Infill density                             | 40%                                                |
| Cross-linking: frequency                   | After every layer                                  |
| Cross-linking: time (per instance)         | 10 s                                               |
| Cross-linking: wavelength                  | 405 nm                                             |
| Cross-linking: distance to center of build | 4 cm                                               |

mainly affect bioink stability and the extrusion process. Cross-linking settings, including frequency, duration, wavelength, and distance to the printed construct at light exposure time, affect the structural integrity of the bioprinted constructs.

The LIVE-DEAD staining kit (Fisher Scientific) is utilized for simultaneous fluorescence staining of live and dead cells to measure the cellular viability of the bioprinted samples. Live cells were stained with Calcein-AM (2  $\mu$ M), and the fluorescence was measured at 494–517 nm, and dead cells were stained with ethidium homodimer-1 (4  $\mu$ M), measured at 528–617 nm. For performing live-dead staining, the construct was washed with PBS. Later, 200  $\mu$ l of the live-dead solution was added to the petri dish containing the bioprinted vascular tissue construct and then stained for 30 min before imaging. Imaging was performed using a Nikon C1 confocal fluorescence microscope. ImageJ (NIH) software was used to evaluate the cellular viability of the bioprinted vascular tissue constructs.

Fig. 2A shows the slide flask containing a bioprinted vascular construct, and Fig. 2B depicts the RPM enclosed inside an incubator used for simulated microgravity experiments. The RPM 2.0, Airbus, YuriGravity was used for simulating microgravity conditions. It consists of two independently rotating frames that rotate at different speeds and directions to randomize the gravity vector. Bioprinted tissue constructs were placed inside the inner rotating platform, and microgravity conditions ( $< 10^{-3}$  g) were achieved. The system supported a maximum load of up to 1.5 kg, and sample holders such as flasks were used. The rotation speed was set between 0 and 20 rpm, depending on experimental requirements, and an on-stage power supply was available to maintain environmental conditions during operation. Prior to the experiment, tissue constructs were transferred into a slide flask and exposed to a simulated microgravity environment over a period of time. The flasks were filled with complete cell media, ensuring the chances of minimal bubble formation during the experimentation.

Post-exposure to simulated microgravity experiments, the bioprinted constructs were fixed to perform biochemical analyses. The fixing procedure involved isolating tissue constructs and placing them in petri dishes with fibroblast endothelial cell basal media, followed by freezing in liquid nitrogen within disposable molds and embedding in OCT. After cooling in a cryostat at  $-20^{\circ}\text{C}$  for 20 min, tissue slices were incubated in Krebs-Henseleit solution (pH 7.4,  $37^{\circ}\text{C}$ ) for 30 min in the dark. The slices were then incubated with 1X PBS at  $37^{\circ}\text{C}$  for 10 min, treated with a probe-working solution at  $37^{\circ}\text{C}$  for 30 min in the dark, and rinsed with 1X PBS. The working probe solution in this study was prepared using dihydroethidium (DHE) staining (Invitrogen, D11347). The DHE probe undergoes oxidation by ROS, producing fluorescent products that can be measured. Finally, the slides were mounted with Vectashield, covered with a coverslip, and stored at  $+4^{\circ}\text{C}$ .



**Figure 2.** Setup for simulated microgravity experiments. (A) Slide flask containing a bioprinted vascular construct; (B) RPM supporting bioprinted tissue constructs housed inside an incubator (NASA KSC Microgravity Simulation Facility).

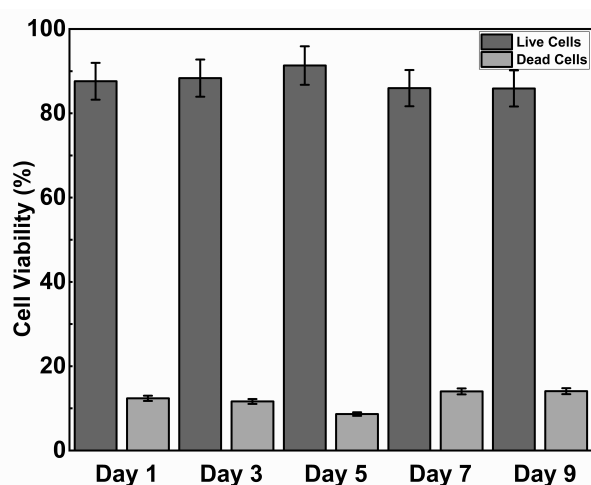
Oxidative stress was characterized by measuring the ROS levels using confocal microscopy with a red fluorescence filter. The bioprinted tissue constructs were embedded in OCT and sectioned into thin slices (5  $\mu\text{m}$ ). The constructs were divided into three groups based on the duration of the exposure to simulated microgravity conditions: control, 24, 48, and 72 h. Control tissue constructs were prepared using the same batch of bioink, maintained in the same type of slide flasks, and cultured under identical conditions (same incubator, media, and time points) but were not exposed to RPM rotation. The samples were cryo-sectioned, and at least four slices from each sample were imaged. ImageJ software was used to quantify fluorescence intensity at different time points for each sample.

## Results

The optimal bioprinting process parameters correspond to a pressure of 40kPa and a temperature of 23.5°C. Pressure gradually increased until a steady extrusion of the bioink is achieved. If the bioink does not extrude, the temperature is raised by 1°C–2°C, followed by a 15-min wait for the ink to reach the new temperature, after which the extrusion is attempted again while simultaneously adjusting the pressure as needed. The highest pressure used is between 70–75kPa. As presented in Table 1, most bioprinting parameters remain constant throughout the process.

Adjustments are made to the bioink pressure to prevent over- or under-extrusion. Parameters such as printhead temperature and print speed are established at the beginning of the printing session while the bioink is settling. A significant challenge lies in the variability of optimal parameters, which can fluctuate between individual prints, making it challenging to achieve reproducibility. As GelMA-based bioink is highly sensitive to environmental conditions, fluctuations in the printing environment during the printing process affect the printing parameters. Printing a six-layer construct in a single well requires approximately 90 s, including three 20s intervals for crosslinking at 405nm (two layers at a time). For a six-well plate, the total printing time is 9 min, assuming uninterrupted operation and excluding the time needed to reposition the build plate between wells. At this time, it is expected that the mechanical properties of the bioink will change, and therefore the printing parameters must be altered to maintain the structural integrity of the bioprinted constructs.

Cell viability of the bioprinted tissue constructs were measured over a 9-day period to determine if the shear rate from the extrusion-based system had any effect on the cell survival rates. Fig. 3 shows the distribution of live and dead cells in the bioprinted tissue constructs from day 1 to 9, highlighting cellular viability above 80%. Cell viability in bioprinted constructs were not assessed in this study beyond 10 days to preserve highly viable tissue constructs for experiments in simulated microgravity, which were conducted over a time period of 72 h.



**Figure 3.** Cellular viability of bioprinted vascular tissue constructs from 1 to 9 days after bioprinting.

Fig. 4 shows the distribution of live and dead cells in the bioprinted tissue constructs for five different time points (day 1 to day 9). Green represents live cells and red represents dead cells. The confocal images show that live cells were predominant at each time point, with minimal cell death occurring after 9 days from bioprinted tissue constructs. The consistent presence of a high population of green cells across all time points indicated that the majority of cells remained viable within the constructs over this time period. Some cell death begins to appear after 9 days, but without significant loss of overall cellular viability. Fig. 4F shows a 3D z-stack confocal fluorescence image corresponding to day 9 after bioprinting. This image representing the spatial distribution of viable and nonviable cells was obtained by stacking multiple layers from top to bottom, providing a 3D representation of the cellular distribution in the bioprinted samples. The image shows that live cells are present on the surface and throughout the inner and deeper layers of the bioprinted constructs, indicating that the constructs are supporting healthy cell growth across the entire thickness of the tissue constructs.

Fig. 5 shows the normalized intensity profiles depicting ROS levels that affect cellular process for bioprinted tissue constructs exposed to simulated microgravity for a duration of 24, 48, and 72 h. In this plot, the baseline ROS level for the control group is set to unity. Fig. 5A shows a box plot representing the distribution of normalized intensity values across all slices ( $n=4$ ) for each time point, while Fig. 5B represents the cumulative effects of simulated microgravity exposure across multiple samples for each exposure time point. The mean values from the individual slices in Fig. 5A were used to calculate the mean ROS levels for each sample, and these sample means were then averaged across replicates to generate Fig. 5B.

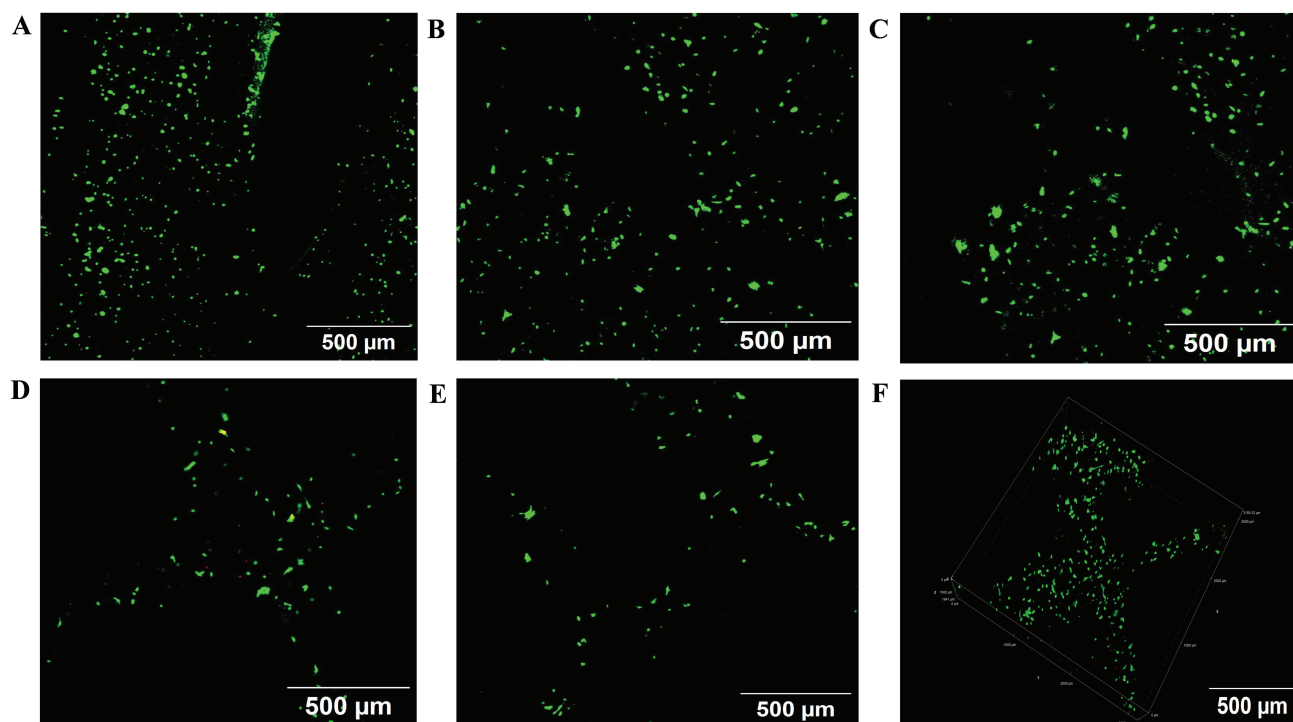
**Table 2.** t-test results comparing ROS levels across time points.

| Comparison       | t-statistic | p-values  | Interpretation     |
|------------------|-------------|-----------|--------------------|
| Control vs. 24 h | -20.74      | 0.0000319 | significant        |
| Control vs. 48 h | -59.20      | 0.0000005 | highly significant |
| Control vs. 72 h | -4.40       | 0.0117    | significant        |

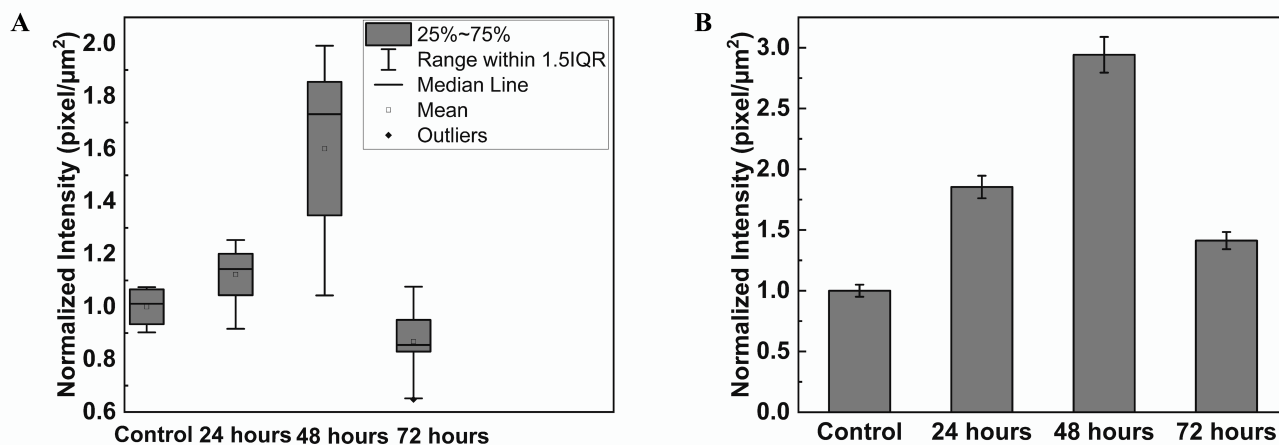
In Fig. 5, the control group showed consistently low ROS levels, indicating minimal oxidative stress observed under normal gravity conditions. After 24 h of exposure to simulated microgravity, a moderate increase in ROS levels was observed, suggesting that cells were beginning to experience oxidative stress. This is possibly due to disrupted gravity-sensing mechanisms like cytoskeletal rearrangement and altered mitochondrial function. The trend of significant increase in ROS levels reflecting maximal oxidative stress damage, and disruption of cells persisted through 48 h of simulated microgravity exposure. However, after 72 h of exposure, ROS levels declined, indicating that many stressed cells had already undergone cell death or entered senescence, while others had adapted by activating antioxidant defenses. Fig. 5A shows a similar trend as Fig. 5B, in which the average mean ROS levels after 24 and 48 h of simulated microgravity exposure were increasing significantly, whereas for 72 h of exposure, there was a decline in the ROS levels.

The statistical significance of our findings was validated through the employment of independent t-tests that compared ROS levels between each time point to the control group. As shown in Table 2, the  $p$ -values for all exposure times fall below the 0.05 threshold, confirming that the increase in ROS levels after 24, 48, and 72 h of simulated microgravity exposure are statistically significant. The highest t-statistics and lowest  $p$ -value were observed at the 48-h exposure mark, identifying it as the most critical time point for oxidative damage. The t-statistic quantifies the difference between two group means in relation to the variability within the data; larger absolute values indicate greater differences. The  $p$ -value represents the probability that this difference arose by chance; lower  $p$ -values suggest the result is statistically significant.

Fig. 6 shows confocal images corresponding to Fig. 5. The red fluorescence represents ROS staining, where increased brightness and intensity correspond to higher levels of oxidative stress encountered by the tissue constructs. In Fig. 6A, minimal red fluorescence is visible, signifying low ROS levels in the bioprinted tissue constructs under normal control conditions. Fig. 6B shows an increase in red fluorescence compared to the control, suggesting an early oxidative stress after 24 h of exposure to simulated microgravity. The fluorescence intensity increases significantly and is more consistent in Fig. 6C, supporting the trend of spike in ROS levels after 48 h of simulated microgravity exposure. In Fig. 6D,



**Figure 4.** Confocal microscopy imaging for visualizing live (green) and dead (red) cells in bioprinted constructs after bioprinting. (A) Day 1; (B) Day 3; (C) Day 5; (D) Day 7; (E) Day 9; (F) Z-stack fluorescent images corresponding to day 9.

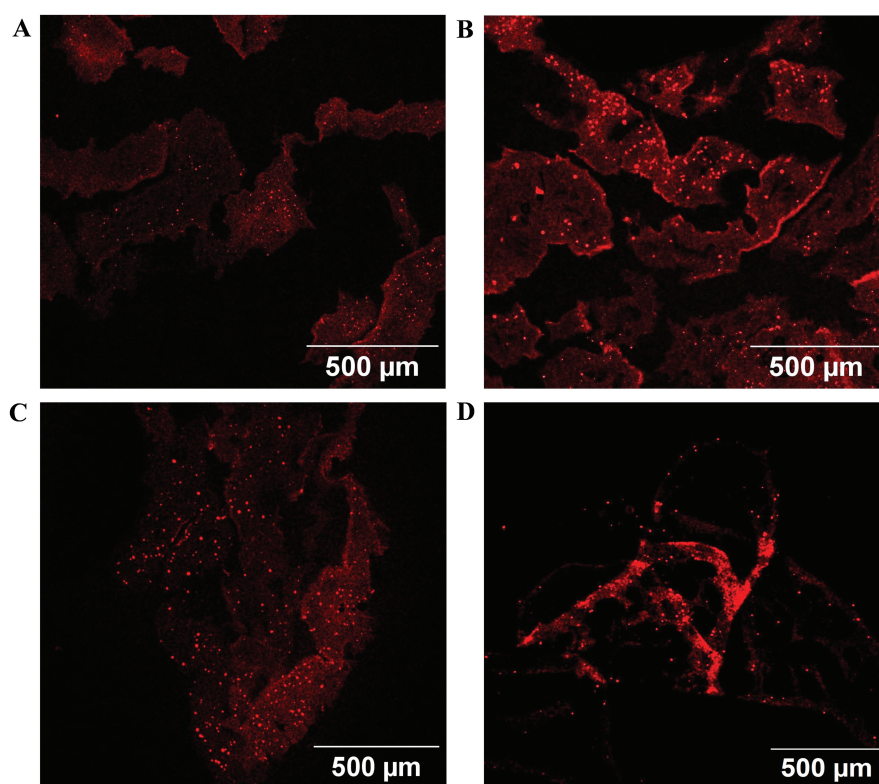


**Figure 5.** Normalized fluorescent intensity profiles for detecting ROS levels in fibroblast cells exposed to simulated microgravity for 24, 48, and 72 h. (A) Bar plot of normalized fluorescence intensity values showing a time-dependent increase in ROS levels; (B) Box plot representing the distribution of normalized fluorescence intensity values across all samples for each condition.

red fluorescence is still visible in samples exposed to 72 h of simulated microgravity but appeared more clustered and less evenly dispersed when compared to samples exposed for 48 h. However, the ROS levels after 72 h of simulated microgravity exposure remained significantly higher than control samples.

## Discussions

The proper extrusion of bioink during the bioprinting process is highly dependent on both temperature and pressure. The pressure is increased incrementally from an initial value of 10



**Figure 6.** Confocal microscopy images showing oxidative stress in the samples: (A) Control; (B) Simulated microgravity exposure for 24 h; (C) 48 h; (D) 72 h.

kPa until a smooth and continuous bioink extrusion is observed. The temperature of the printhead should initially be at or above the desired print temperature while the ink is settling in the cartridge. This helps facilitate a uniform temperature profile change from 37°C to the desired printhead temperature. It is critical to allow 15 min for the ink to completely adjust to the change in temperature. Once the bioink reaches the desired temperature, continuous extrusion is achieved by changing the pressure. This optimization process of temperature and pressure control is crucial to ensure smooth bioink extrusion. The temperature affects the viscosity of the bioink, with higher temperatures generally lowering viscosity, making it easier for the bioink to flow. However, too high temperatures will result in low cross-linking density and weak structural integrity. The optimal temperature of the printhead can be determined by conducting a viscosity flow sweep of the bioink at incremental temperatures using a rheometer. Specifically, temperatures expressing viscosity profiles that indicate both stability and reversibility are optimal for bioprinting. These temperature thresholds indicate sufficient viscosity to maintain desired shape fidelity after printing (gel-like structure) but still express optimal fluidity for smooth extrusion through the print nozzle. Extrusion-based bioprinting involves forcing bioink containing

cells through a nozzle, which can generate significant shear stress without proper control of bioprinting parameters. The bioprinted constructs maintained a high level of cellular viability, and the mechanical force during bioprinting can damage cells, leading to a slight reduction in the viability of cells, as observed in Fig. 3.

Fig. 4 shows a uniform distribution of cells in bioprinted constructs after bioprinting, which demonstrates the ability of the bioprinting process to support cell survival and maintain structural integrity by controlling the printing parameters. The uniform dispersion of cells within the bioink before printing ensures that the bioprinted constructs have a uniform cell distribution, contributing to viability and functionality, as observed in Fig. 4.

The normalized fluorescence intensity values in Fig. 5 directly reflect the ROS levels manifested in cells. Dysregulation of ROS production under simulated microgravity environment has been implicated in the pathogenesis of many diseases and aging. In Fig. 5A, the control group shows consistently low ROS levels with minimal variability, indicating that the cells experienced minimal oxidative stress. In contrast, the bioprinted samples exposed to simulated microgravity for 24 and 48 h show consistently elevated ROS levels compared to

the control. The highest ROS levels manifested in cells after 48 h of simulated microgravity exposure also expressed the largest variability, as evident in Fig. 5A. This could represent some cellular heterogeneity at this critical time point, where cells may activate antioxidant responses to protect against the higher levels of oxidative stress (Berardini et al., 2023).

Fig. 5B summarizes the trends in oxidative stress for various exposure time points: a progressive increase from 24 to 48 h is followed by a decrease after 72 h, highlighting the fact that the peak oxidative stress after 48 h of simulated microgravity exposure is followed by partial recovery or adaptation after 72 h of exposure. These findings are supported by several prior studies using simulated microgravity that reported that cardiomyocytes and osteoblasts show high oxidative damage at 48 h (Morabito et al., 2020; Guarnieri et al., 2021). Unlike the previous studies, which used a 2D cell culture model, the results presented in this paper is the first to demonstrate the temporal oxidative stress characteristics using a 3D bioprinted vascularized tissue model. Studies have also shown that early microgravity exposure disrupts cytoskeletal structure and redox balance, leading to the start of oxidative stress, which is consistent with our findings after simulated microgravity exposure of bioprinted tissue constructs for 24 h (Ran et al., 2016; Guarnieri et al., 2021).

The confocal images in Fig. 6, when interpreted alongside the quantitative data in Fig. 5, suggest a dynamic cellular response to oxidative stress induced by simulated microgravity. While ROS levels increase over time, the spatial distribution patterns particularly the clustered fluorescence observed at the end of 72 h of exposure indicate a shift in cellular state such as activation of defense pathways or onset of damage-related processes. Previous studies have shown that simulated microgravity can increase ROS levels and activate protective stress responses such as an Nrf-HO-1 pathway as a cellular defense mechanism (Zhang et al., 2025). This adaptive mechanism explains the altered distribution pattern seen after exposure for 72 h, where some cells have entered a protective phase.

Additionally, microgravity has been associated with disrupted cytoskeletal organization and impaired intercellular signaling in fibroblast populations, as reported by Cialdai et al. (2022), which contribute to the uneven ROS levels observed after 72 h of simulated microgravity exposure. This irregular fluorescence pattern could reflect localized oxidative damage or uneven cellular stress adaptation. Interestingly, antioxidant interventions such as salidroside have been reported to reduce microgravity-induced oxidative stress and preserve cellular function (Wang et al., 2024), highlighting potential therapeutic strategies for counteracting the damage. These findings underscore the importance of the 48-h duration of simulated microgravity exposure as a potential tipping phase for oxidative stress responses where the balance between damage and adaptation may be most pronounced.

## Conclusions

The findings of this study demonstrated that the bioprinted vascular tissue constructs developed using GelMA-based bioink maintained high cellular viability and structural integrity. The functionality of the bioprinted constructs was affected by exposure to simulated microgravity due to the manifestation of higher levels of oxidative stress. A significant increase in ROS level was observed after exposure to a simulated microgravity environment after 48 h, implying that the more prolonged microgravity exposure induced oxidative stress in bioprinted tissues at that time point. This underscores the critical need for enhanced strategies, such as antioxidant incorporation or advanced culture systems, to mitigate oxidative stress in microgravity conditions. These insights contribute to understanding cellular responses in space-like environments and have implications for developing therapies for oxidative stress-induced conditions for Earth-based applications.

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## Author Disclosure Statement

Sampada Koirala, Bela Perdomo, Brian Billings, Dylan Welch, Roshan Vijayakumar, Meghana Nelli, Caroline Moore, Caleb Phillips, Isscca Hall Burns, Kunal Mitra. Conceptualization, KM. Methodology, SK, BP, and BB. Investigation, SK, BP, BB, DW, RV, MN, CP, IB. data analysis, SK, RV, MN, CM. writing—review and editing, SK and KM. supervision and project administration, KM. All authors have read and agreed to the published version of the manuscript.

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