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Hypothesis

MRTF May Be the Missing Link in A Multiscale Mechanobiology Approach toward Macrophage Dysfunction in Space

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Abstract

Macrophages exhibit impaired phagocytosis, adhesion, migration, and cytokine production in space, hindering their ability to elicit immune responses. The combined effect of spaceflight microgravity and radiation on macrophage dysfunction is multiscale and multifactorial in nature. However, spaceflight and simulated microgravity experiments often take single-scale approaches. This hypothesis and theory paper reanalyzes research in the macrophage spaceflight response across multiple scales, from the molecular, intracellular, extracellular, to the physiological. Here, we introduce the time-dependent, mechanotransducive MRTF-A/SRF pathway, which depends on intracellular localization, to elucidate seemingly contradictory macrophage responses across time scales. We discuss the MRTF-A/SRF pathway dependence on the actin cytoskeleton/nucleoskeleton, microtubules, mechanosensitive membrane proteins, hypoxia, oxidative stress, and crosstalk with other pathways and cells. By adopting a multiscale perspective, this paper identifies potential mechanisms for adverse macrophage responses and strengthens the connection between microgravity, mechanobiology, and the spaceflight immune response. Finally, we hypothesize MRTF involvement and complications in treating spaceflight-induced cardiovascular, skeletal, and immune disease.

Keywords: mechanobiology; microgravity; macrophage; multiscale; MRTF; radiation

1 INTRODUCTION

1.1 Multiscale approaches

Multiscale approaches in mechanobiology consider molecules, single cells, tissues, and organs, including each of their varied responses across time scales, to resolve complex interactions between biology and mechanics (Fritzsche, 2020). Similarly complex, the combined environmental effect of spaceflight microgravity and radiation has been given a multiscale mechanobiology approach for cardiovascular disease (Basirun et al., 2021) and muscle/bone loss (Deymier et al., 2020), but not for immune dysregulation. Yet current immune studies in microgravity vary in scale from drop-towers to ballistic flights to long-term spaceflight (seconds to minutes to months), reviewed in detail by ElGindi et al. (2021), or microgravity is simulated for a few days in 3D random positioning machines (3D-RPM) and rotating wall vessel bioreactors (RWV), where constant rotation time-averages the gravity vector to be negligible (Hammond and Hammond, 2001).

Macrophages (Mϕ), a primary immune cell type, are commonly given multifactorial analysis (Cess and Finley, 2020; Orsini et al., 2021) because their phenotype is affected by a dynamic balance of extracellular cytokine signaling, intracellular crosstalk, cell-cell interaction with immune cells, and mechanical and physiological environment (Finch-Edmondson and Sudol, 2016; Decano and Aikawa, 2018). These factors are space- and time-dependent, and thus differential changes observed across experimental timescales were often interpreted as an adaptation to microgravity (Meloni et al., 2006; Paulsen et al., 2015; Ludtka and Silberman et al., 2021). Instead of such broad interpretations, refined conclusions are necessary for safe, effective treatment of spaceflight diseases such as immune dysregulation (Crucian

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et al., 2018), cancer progression (Kim et al., 2021), circadian rhythm disruption (Simmet, 2013), and accelerated atherosclerosis (Meerman et al., 2021). For example, blood-circulating monocytes are recruited as pro-inflammatory Mϕ toward atherosclerotic lesions because of many factors, including adherence-proteins (Yang et al., 2005), motility, (Mukherjee et al., 2022), reactive oxygen species (ROS) (Y. Wang et al., 2014), and radiation (Patel, 2020), all of which are regulated by spaceflight.

Here, we apply a multiscale approach incorporating microgravity, mechanotransduction, radiation, and crosstalk to propose mechanisms for the most well-studied Mϕ phenotype changes in space: pro/anti-inflammatory activation, morphology, migration, and phagocytosis. We briefly describe individual spaceflight effects in increasing order of space and time (Figure 1). To address knowledge gaps, we introduce emerin—a putative gravi-sensitive nuclear envelope protein (Aventaggiato et al., 2020)—, novel mechanisms for arginase-1 (*ARG1*) microgravity regulation, and, most notably, a novel scale in the multiscale space milieu via the myocardin-related transcription factor/serum-response factor (MRTF-A/SRF) pathway. MRTF-A/SRF reinforces space studies that would otherwise have seemingly contradictory conclusions regarding suppression or activation of the pro-inflammatory (M1) response of the uniquely mechano-regulated Mϕ cell type.

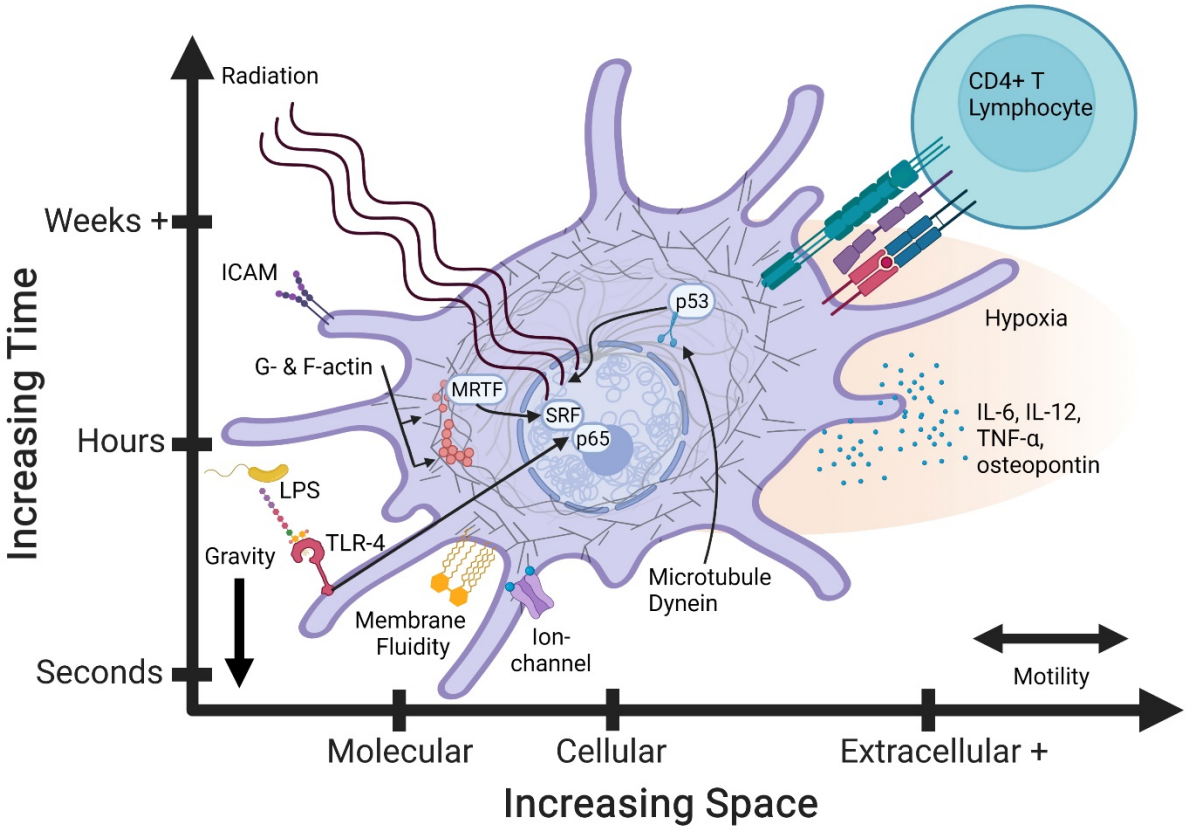

Figure 1. Some Mϕ spaceflight effects require more time or more space. An overview of the altered spaceflight exposome (gravity, cytoskeleton, intracellular transport, hypoxia, radiation, intercellular signaling) and hypothetically relevant sensors and effector proteins: (LPS—lipopolysaccharide, TLR-4—toll-like receptor 4, ICAM—intercellular adhesion molecule, G-actin—globular actin, F-actin—filamentous actin, microtubules, dynein, p53—tumor protein P53, MRTF—myocardin-related transcription factor, SRF—serum response factor, NF-κB/p65—nuclear factor kappa-light-chain-enhancer of activated B cells, IL-6—interleukin 6, IL-12, TNF-α—tumor necrosis factor-alpha, osteopontin). Spatial variation occurs across molecular, cellular, and physiological scales (increasing space). Time variation occurs from seconds in microgravity to months from long-term radiation (increasing time). Effects are not mutually exclusive and may interact at multiple scales, i.e., microgravity first acts alone and later acts in conjunction with radiation. Created with BioRender.com.

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1.2 MRTF-A in Mϕ pro-inflammatory activation

Mϕ pro-inflammatory activation occurs firstly in a chemical and secondly a mechanotransducive phase lasting 0-3 hours and 4-24 hours respectively (Jain and Vogel, 2018). In the first stage, activation of surface receptors induces NF-κB/p65 nuclear translocation. Secondly, actin polymerization modulates cytokine transcription/secretion via transport of MRTF-A to the nucleus (Olson and Nordheim, 2010; Zhou et al., 2021), where it slowly accumulates over three hours and associates with serum response factor (SRF). MRTF-A mechanosensitivity is well-studied; if mechanical force affects polymerization of G-actin to filamentous-actin, then G-actin-bound MRTF-A is released and transported:

F-actin ↔ G-actin ↔ MRTF-A ←| Nuclear Envelope |→ SRF → Transcription of target genes

SRF target genes include interleukin 6 (IL-6), IL-1β, and inducible nitric oxide synthase (iNOS) (Jain and Vogel, 2018; Yang et al., 2020). Downstream effects include the secretion of pro-inflammatory cytokines IL-6, IL-12, and interestingly, tumor necrosis factor-α (TNF-α) (Jain and Vogel, 2018)—thus TNF-α secretion and *TNF-α* expression (p65 promoted) are regulated by distinct mechanisms in Mϕ.

Many studies, reviewed by Sun et al. (2021), have found the Mϕ NF-κB inflammatory pathway to be unaffected by microgravity. If not caused by NF-κB, then what is the mechanism of Mϕ phenotypic change? The effect of microgravity on the MRTF-A/SRF pathway has not been explored in Mϕ and has been rarely explored in other cell types. For example, Chang et al. (2012) analyzed astronaut T-cell transcriptomic profiles, finding the majority of downregulated genes to be promoted by SRF. We emphasize the importance of research in Mϕ because the cell type is implicated in diseases associated with spaceflight, such as circadian clock disruption (Shirato and Sato, 2022)—where Mϕ circadian clock components that regulate the timing of phagocytosis and motility are promoted by MRTF-A (Kitchen et al., 2020; Xiong et al., 2021). MRTF-A is also overexpressed in atherosclerotic plaque-associated Mϕ (An et al., 2019). Lastly, it is important to note that MRTF-A/SRF mediates actin and myosin gene expression (Guenther et al., 2019). We interpret this as a delayed feedback loop for cytoskeletal remodeling, which may be a mechanism for long-term adaptation of Mϕ in microgravity.

2 MULTISCALE ANALYSIS

2.1 Microgravity-induced mechanical unloading

Mechanical factors such as shear stress, extracellular matrix (ECM)/tissue stiffness, and spatial confinement (Jain et al., 2019) correlate to immune regimes that govern Mϕ phenotype throughout the body. Innate immune system function necessitates Mϕ motility and phagocytosis, both of which require rapid cytoskeletal remodeling (Orsini et al., 2021). Likewise, microgravity—which in drop towers and parabolic flights is studied in second-long intervals—induces rapid cytoskeletal restructuring via actin depolymerization, but Mϕ repolymerizes actin and corrects it within minutes (Thiel et al., 2019), potentially due to feedback loops associated with the cellular level of actin polymerization. For example, the nucleoskeleton, which regulates and remodels chromatin (Venit et al., 2021), is similarly restructured, the result being the modulation of mechano-sensitive genes.

Additionally, the Mϕ cytoskeleton is physically linked with the plasma membrane. This linkage mediates motility and phagocytosis (Liu et al., 2020). Although less studied in microgravity, there is evidence presented by Kohn et al. (2017) that microgravity increases cellular lipid membrane fluidity. This may disrupt lipid rafts in microgravity, for instance, allowing free diffusion of caveolin-1 (Le Roux et al., 2019)—a crucial protein for Mϕ phagocytosis (Li et al., 2005; Rubio et al., 2018). Also, membrane ion channels often have importance to inflammation, and are rapidly sensitive to membrane tension/fluidity, but are rarely studied in Mϕ under microgravity. Yet, the two best-studied mechano-sensitive Ca^{2+} ion-channels, transient receptor potential vanilloid 4 (TRPV4) and Piezo1, vary in activation responses to cytoskeletal structure, substrate stiffness/topology, and membrane tension/fluidity (Bryant et al., 2017; Botello-Smith et al., 2019; Romero et al., 2019; Sun et al., 2019; Krizaj et al., 2020; Orsini et al., 2021; Sianati et al., 2021). Another tension-sensitive H^+ channel, Hv1, is responsible for inducing superoxide production for the Mϕ oxidative burst reaction after phagocytosis (Ramsey et al., 2009), and interestingly, the channel has a mechanical history of up to five minutes (Pathak et al., 2016), which may have ramifications on microgravity platforms with cyclic loading e.g. 3D-RPM or RWV, or

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parabolic flight with a gravity period of ~20 seconds. Because the plasma membrane quickly responds to mechanical forces, it may play a role in the Mϕ oxidative burst reaction, which rapidly adapts to microgravity (Adrian et al., 2013; Thiel et al., 2017).

2.2 Mechanotransduction

Gene expression is often studied on the timescale of hours in simulated microgravity bioreactors, which oscillate the gravity force usually around 10-15 rpm. Expression is not only induced by biochemical pathways but directly from the physical linkage of the cytoskeleton to the nucleoskeleton (Jaalouk and Lammerding, 2009). Remarkably, Guilluy et al. (2014) demonstrated nuclear stiffening under cyclic (9 /min) mechanical force as small as 35 pN (near the weight of a Mϕ cell). They identified nuclear lamin protein emerin to be involved independently from the nucleoskeleton. We identify emerin to be a potential confounding cause of nuclear stiffness discrepancies across simulated/spaceflight microgravity platform factors—e.g. rotation frequency, substrate stiffness, or topology. Table 1 compares cell stiffness, migration, and filamentous actin (F-actin) levels across simulated microgravity platforms and culture method, which vary in substrate rigidity, cell adhesion, or extracellular matrix (ECM). Cells cultured on rigid substrates at 10-15 rpm (close to 9 /min where emerin nuclear stiffening was observed) are more motile, stiffer, or exhibit greater actin polymerization (Mao et al., 2016; Thompson et al., 2020; Wubshet et al., 2021), apparently contradicting general findings of spaceflight microgravity studies. Moreover, emerin regulation of the cyto-/nucleo-skeleton is known to modulate MRTF-A levels in the nucleus (Sidorenko et al., 2022).

Table 1. Simulated microgravity alters nuclear and cytoskeletal structural dynamics in various cell types and culture methods. Boldened results indicate concordance with observed spaceflight microgravity motility studies. The nucleus is the stiffest organelle and contributes the most to cellular stiffness (Qi et al., 2016). Increased actin polymerization generally increases nuclear size and stiffness via musculoskeletal remodeling (Liu et al., 2019), thus reducing cellular motility (McGregor et al., 2016). Generally, cell motility is reduced in spaceflight and simulated microgravity across various cell types (Meloni et al., 2011).

Cell Type	Platform	Culture Method	Results	Study
J-111 monocyte	**3D-RPM, 60 rpm**	**Chamber slides (Lab-Tek)**	**↓ F-actin** **↓ Cell migration**	**(Meloni et al., 2006)**
Human breast epithelial cell	**3D-RPM, 2 rpm**	**Cell culture flask (Fisher)**	**↑ Nuclear volume**	**(Neelam et al., 2020)**
MLO-Y4 Osteocyte	**RWV, 15 rpm**	**Cell Rolling Tube (Thermo Scientific Forma™)**	**↑ Nuclear volume** **↓ F-actin polymerization**	**(Yang et al., 2018)**
Human osteoblast	3D-RPM, ~10 rpm	Adherent cell culture	↓ Cell stiffness **↓ F-actin**	(Wubshet et al., 2021)
Rat bone marrow mesenchymal stem cell	RWV, 10 rpm	2D cell culture slide	**↑ Cell stiffness** ↑ F-actin polymerization	(Mao et al., 2016)

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Mouse mesenchymal stem cell	RWV, 15 rpm	SlideFlasks (2D plated cells)	↓ Nuclear stiffness (not significant) ↓ F-actin (not significant)	(Thompson et al., 2020)

After a few minutes in microgravity, microtubule arrangement is disrupted (Papaseit et al., 2000) and in the span of five days, microtubules are shorter and wavier in Mϕ (Nabavi et al., 2011). Consequently, microtubule disruption induces the p38 mitogen-activated protein kinase (MAPK) pathway (Cuenda and Rousseau, 2007); thus we hypothesize that microtubule disruption is the cause of p38 MAPK induction, and further upregulation of *ARG1* observed in Mϕ in simulated and spaceflight microgravity (Wang et al., 2015; Ludtka and Silberman et al., 2021). In fact Mϕ *ARG1* expression is induced by perturbing microtubules via chemical methods, yet is not affected by chromatin remodeling nor by ECM stiffness (Meizlish, 2021). However, p38 MAPK induction is linked to mechanosensitive membrane proteins (Cuenda and Rousseau, 2007; Ludtka and Silberman et al., 2021). The timescale difference between membrane proteins and microtubule arrangement could be why Mϕ arginine level modulation varies between short- and long-term spaceflight (Thiel et al., 2021).

2.3 Intracellular localization and transport

MRTF-A/SRF cytoskeletal mechanotransduction is a slow process that takes up to four hours vs. ~10 minutes for NF-κB (Bagaev et al., 2019). We hypothesize that delayed mechanotransducive pathways are a cause for experimentally observed "adaptations" to microgravity and that inconsistencies observed across studies (Table 2) are time-dependent and pathway-specific. For example, cytokine expression/secretion of pro-inflammatory IL-6/IL-12/IL-1β is significantly downregulated after 4-24 hours, concordant with our theory that actin disruption in microgravity inhibits the MRTF-A/SRF pathway. Interestingly, if microgravity is turned off post-48 hours, then cytokine expression/secretion appears to recover (Table 2). Likewise, there is no time dependence of NF-κB-dependent TNF-α expression/secretion as it is consistently downregulated in both simulated and spaceflight microgravity. We also consider an alternative mechanotransducive pathway, p38 MAPK, in two studies where the data are available (Table 2), which may explain their inconsistency with IL-6 and IL-12 expression/secretion as activation of p38 MAPK increases *IL-6* and decreases *IL-12* expression (Wang et al., 2015). Unlike MRTF-A, p38 (MAPK-C/EBPβ) is actively transported to the nucleus within minutes via dynein on microtubules (Gong et al., 2010; Maik-Rachline et al., 2020).

Table 2. Mϕ stimulation cytokine responses over time in microgravity

Boldened results indicate a reduction in pro-inflammatory cytokines TNF-α/IL-6/IL-12/IL-1β, and thus concordance with our theory of microgravity-based MRTF inhibition. Anti-inflammatory cytokines include IL-10. Protocols between studies varied the order between pro-inflammatory stimulation and microgravity.

Cell Type	Platform	Culture Method	Time after stimulation	Results	Study
U937 differentiated to Mϕ after RWV	RWV, 18 rpm	10-mL RCCS-D bulk vessels (Synthecon)	**1-3 hr** after 12 hr differentiation and 72 hr RWV	**↓ IL-6 secretion, expression, exacerbated over time** **↓ *TNF-α* expression** **↓ TNF-α secretion, exacerbated over time** ↓ p38 MAPK pathway	(Wang et al., 2020)
RAW 264.7 & primary mouse Mϕ	RWV, unspecified rpm	Adherent microcarrier beads	**4 hr** after 24 hr RWV	**↓ IL-6, IL-12 secretion** **↓ TNF-a secretion** Unchanged p38 MAPK pathway	(C. Wang et al., 2014)

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Primary mouse Mϕ	RWV, 12-25 rpm	Adherent microcarrier beads	**4 hr** after 24 hr RWV	↑ IL-6 expression and concentration **↓ IL-12 subunit B expression** ↑ p38 MAPK pathway	(Wang et al., 2015)
			24 hr after 24 hr RWV	**↓ (less significant) IL-12 subunit B concentration** ↑ p38 MAPK pathway	
RAW 264.7 murine Mϕ	RWV, 14 rpm	10-mL RCCS-D bulk vessels (Synthecon)	**48 hr** after 48 hr RWV	**↓ IL-6, IL-12 secretion** **↓ TNF-α, NO secretion**	(Hsieh et al., 2005)
Human blood monocyte stimulated with LPS	Spaceflight	*In vivo*, then whole blood cultured, and stimulated	under 1g **48 hr,** after ~350 hr spaceflight	↓ IL-6 expression ↑ IL-1β expression **↓ *TNF-α* expression** ↓ IL-10 expression	(Crucian et al., 2011)
Mouse splenocyte stimulated with LPS	Spaceflight	*In vivo*, then flat-bottom plated, and stimulated	under 1g **48 hr,** after ~312 hr spaceflight	↑ IL-6 secretion IL-12 (ns) **↓ TNF-α secretion** ↑ IL-10 secretion	(Baqai et al., 2009)
RAW 264.7 murine Mϕ	RWV, 14 rpm	Adherent microcarrier beads	72 hr RWV after **48 hr** of stimulation	IL-6 (ns) ↑ IL-12 secretion **↓ TNF-α secretion** ↑ IL-10 secretion	(Ludtka and Moore et al., 2021)

Our identification of MRTF-A/SRF pathway inhibition is the first time that time-dependent Mϕ cytokine profiles have been linked to microgravity. Not just cytokine profiles, but also a previous experiment (Hsieh et al., 2005) (Table 2) showed reduced nitric oxide (NO) secretion. In correlation, MRTF-A/SRF promotes iNOS, a producer of NO (Yang et al., 2020), which is essential for killing pathogens after phagocytosis. For this reason, we also conjecture that MRTF-A is a factor in impaired Mϕ phagocytosis in microgravity. MRTF-A-promoted genes involved in phagocytosis include caveolin-1 (Krawczyk et al., 2015) and intercellular adhesion molecule 1 (*ICAM-1*) (Zhong et al., 2021; Huang et al., 2022, p. 6). ICAM-1 is a transmembrane protein found clustered in lipid rafts (Tilghman and Hoover, 2002) and anchored to the actin cytoskeleton (Schaefer et al., 2014). However, the regulation of MRTF-A on *ICAM-1* is not consistent across cell type and is unclear in Mϕ (Fang et al., 2011; Hayashi et al., 2015). Additionally, the effect of microgravity on ICAM-1 regulation is controversial, varying between cell types (Paulsen et al., 2014; Tauber et al., 2017; Buravkova et al., 2018). For Mϕ, it is seemingly time-dependent (Table 3), but no microgravity-linking mechanism has been identified yet.

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Table 3. ICAM-1 surface expression in differentiated and non-differentiated Mϕ/monocytes. Simulated and spaceflight microgravity modulated U937 and human Mϕ ICAM-1 surface levels, but did not affect non-differentiated monocytes, even transcriptionally. Note, a microgravity phase of parabolic flight lasts 20 seconds, not enough time for differential transcription, thus differential surface expression of ICAM-1 may be attributed to membrane/cytoskeletal dynamics or other post-transcriptional factors.

Cell Type	Platform	Culture Method	Time	Results	Study
Non-differentiated Monocytes, both stimulated and non-stimulated during flight					
U937 human monocyte	Parabolic flight	Nutrimix bag (B. Braun Melsungen)	20 s	No change in ICAM-1 surface expression	(Paulsen et al., 2015)
U937 human monocyte	Sub-orbital rocket	Plastic Syringe	6 min	No change in ICAM-1 mRNA levels	(Paulsen et al., 2015)
Differentiated Monocytes/Mϕ					
U937 human Mϕ-like monocyte	Parabolic flight	Nutrimix bag (B. Braun Melsungen)	20 s	↑ Slight ICAM-1 surface expression	(Paulsen et al., 2015)
Human primary Mϕ and U937 human Mϕ-like monocyte	RWV, 60 rpm	Serological pipette	24-120 hr	↑ Surface ICAM-1 **trending down (not significant) over time**	(Paulsen et al., 2015)
U937 human Mϕ-like monocyte	Geocentric orbit	Polycarbonate slide	120 hr	↑ Surface ICAM-1 Severe disturbance of the cytoskeleton	(Paulsen et al., 2014)
Primary human Mϕ	Low-earth orbit	Polycarbonate slide	264 hr	↓ Surface ICAM-1 No disturbance of the cytoskeleton	(Tauber et al., 2017)
			720 hr	↓↓ Surface ICAM-1 Altered cytoskeletal architecture	

NF-κB-dependent induction of Mϕ ICAM-1 levels off after ~12 hours (Zhong et al., 2021). Knowing that Mϕ activation is metabolically regulated by epigenetic "brakes" (Ivashkiv, 2013), we posit that MRTF-A is a delayed regulator of ICAM-1 expression in Mϕ. Similarly, Hayashi et al. (2015) found that in vascular endothelial cells, nuclear MRTF-A binds to NF-κB/p65, inhibiting p65 promotion of *ICAM-1*. Thus, the involvement of NF-κB and MRTF-A/SRF pro-inflammatory pathways may explain the inconsistency across cell types about ICAM-1 expression in microgravity. For example in Table 3, we compare Mϕ to non-differentiated monocytes, a cell type that exhibits unchanged ICAM-1 levels during microgravity flights. Correspondingly, microarray analysis of these monocytes in microgravity has shown that only two pathways are weakly altered after six minutes of pro-inflammatory stimulation: NF-κB, and the Epstein-Barr virus infection (Paulsen et al., 2015), which is related to the nuclear transport and function of p65 (Morrison and Kenney, 2004). These two pathways correlate with the first stage of pro-inflammatory activation. Comparatively in Mϕ and pre-differentiated monocytes, relative surface ICAM-1 levels trended downwards with time (Table 3). This may be interpreted either as a resurgence of MRTF-A as the actin cytoskeleton recovers after 24 hours or as a separate, unknown long-term mechanism for *ICAM-1* downregulation.

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2.4 Hydromechanics of simulated and spaceflight microgravity

Fluid shear, buoyant convection, convective mixing, and the effects of altered chemical/gas diffusion are commonly assumed to be negligible in simulated and spaceflight microgravity but are still part of the multiscale space milieu (Poon, 2020; An and Lee, 2022). For instance, Mϕ ROS production is quickly responsive to shear forces, which are observed in RPM bioreactors that rotate randomly (Brungs et al., 2019). Moreover, hydromechanical transport is a factor of altered phenotype of Mϕ when they are cultured on 2D vs. 3D substrate (Bhattacharya et al., 2020). Therefore, some microgravity hydrodynamic environments may exhibit altered chemical/gas diffusion, conferring local Mϕ hypoxia in culture. Overall effects may include activation of the p38 MAPK pathway (Paardekooper et al., 2018)—a pathway that exhibits mixed responses in simulated microgravity (Table 2). Another potential effect is altered metabolism, as glycolysis results in lactic acid accumulation in culture, thus stimulating pro-inflammatory cytokine expression in Mϕ (Shi et al., 2021).

Hypoxia-inducible factors (HIF) are induced not only by hypoxic environments, but also by inflammatory cytokines such as IL-6, IL-18, and TNF-α in Mϕ (Vogel et al., 2019). HIF-1α is well studied in microgravity: Ludtka and Moore et al. (2021) cultured Mϕ on adherent microbeads in RWV and observed no significant change of *HIF-1α* expression in Mϕ, yet relatedly, observed upregulation of vascular endothelial growth factor (VEGF) secretion and downregulation of *TNF-α*. It is unclear whether this is caused by hypoxia, ROS, or mechanotransducive pathways. For example, the ERK/MAPK signaling pathway induces VEGF secretion across many cell types (Kim et al., 2009; Guo et al., 2020). Interestingly, myofibroblast differentiation is suppressed in hypoxia due to HIF-1α dependent inhibition of RhoA, a key remodeler of the actin cytoskeleton, overall hindering the MRTF/SRF pathway (Leinhos et al., 2019). Furthermore, hypoxia upregulates Mϕ expression of ICAM-1 (Beck-Schimmer et al., 2000) likely in a p53 or NF-κB dependent manner (Gorgoulis et al., 2003). Generally, hypoxia polarizes Mϕ toward anti-inflammatory phenotypes (Ke et al., 2019). Thus we hypothesize that hypoxia and microgravity act in concert to suppress Mϕ pro-inflammatory activation.

2.5 Radiation and oxidative stress

The timespan of space radiation study ranges from weeks to months vs. microgravity study timespans of minutes to days. In contrast to hypoxia, we hypothesize that low-dose space radiation counteracts the effect of microgravity on Mϕ immune function. The immunomodulatory effect of radiation is dosage-dependent and depends on a multitude of factors including DNA damage, ROS generation, and modulation of inflammation pathways. A review in a cancer radiotherapy context by Wu et al. (2017) acknowledges that low-dosage radiation (comparable to spaceflight-relevant dosage) generally induces anti-inflammatory (M2) activation—possibly by inactivation of p38 MAPK—but high doses induce pro-inflammatory (M1) activation, possibly by activation of p53—a well-studied transcription factor that stimulates DNA repair or apoptosis. Similar to p38 MAPK, p53 is transported by dynein on microtubules (Giannakakou et al., 2000).

Mϕ abrogation of phenotypic disorder observed in space may be misattributed to adaptation to microgravity instead of the long-term effects of radiation. For instance, an apparent reversal of ARG1 (Thiel et al., 2021) and surface ICAM-1 expression between 11-30 days in orbital spaceflight (Table 3) may be caused by inactivation of p38 MAPK. A competing mechanism may be membrane-based: oxidative stress is caused by DNA damage and other radiation mechanisms i.e. upregulation of NADPH oxidase (NOX) causes ROS production (Sakai et al., 2018). ROS-based lipid peroxidation causes membrane fluidity reduction (de la Haba et al., 2013)—opposite to the effect of microgravity on fluidity. Nonetheless, there is evidence that space radiation alone is not significant for ROS production, but requires microgravity as a synergistic potentiator (Smith et al., 2012; Ran et al., 2016; Gomes et al., 2018). Considering the synergism between microgravity and radiation, it is possible that they involve MRTF-A and p65 (NF-κB) respectively—both of which form a complex to promote ROS-producing *NOX4* (Rozycki et al., 2016). Relatedly in vascular endothelial cells, oxidized low-density lipoprotein (oxLDL) causes cellular acetylation of MRTF-A promoting nuclear translocation and modulation of *ICAM-1* expression (Huang et al., 2022). Thus, chronic ROS generation could cause the apparent abrogation of ICAM-1 in spaceflight.

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2.6 Intercellular and physiological crosstalk

Mϕ dysregulation translates to impaired interaction with other immune cells. For example, reduced surface ICAM-1 expression in spaceflight (Table 3) can hinder Mϕ adhesion to $CD4^+$ T lymphocytes and subsequent activation (Lin et al., 2020). T lymphocyte interaction and overall immune reaction may also be suppressed by slowed Mϕ migration and motility (Meloni et al., 2006). Another example, Wang et al. (2020) found that simulated microgravity results in mouse gut microbiota dysbiosis and suppression of the p38 and ERK MAPK pathways in intestinal Mϕ. p38 and ERK was rescued by probiotics, thus microgravity may mechanoregulate the microbiota-immune axis. Lastly, Mϕ are mediators of intercellular signals. As observed in coculture by Fu et al. (2019), radiation-induced apoptosis signaling is propagated by Mϕ, potentially increasing tissue damage. Damaged-cell intercellular signaling is enough to stimulate Mϕ differentiation/activation, regardless of Mϕ irradiation state.

Monocyte/Mϕ differentiation also depends on both microgravity and radiation. Shi et al. (2021) observed that microgravity suppresses differentiation of Mϕ to either pro-inflammatory or anti-inflammatory phenotype; yet, Coates et al (2008) observed that radiation augments Mϕ differentiation. Earlier, we have hypothesized that—regarding the innate immune response—radiation counteracts microgravity, but the effect of microgravity and radiation appears additive regarding bone degeneration because of increased fusion of monocyte/Mϕ to form multinucleated osteoclasts (Shanmugarajan et al., 2017). Mϕ originating in bone marrow also communicate locally with other cells: osteopontin, a versatile protein involved in bone cell migration, is promoted in osteoblasts under microgravity (Smith, 2020). Osteopontin also acts as a cytokine for Mϕ (Fantuzzi, 2003) generally promoting phagocytic activity (Schuch et al., 2016). Mϕ produces osteopontin when stimulated with anti-inflammatory IL-18 and IL-10, which are both upregulated by ROS and mechanical stress (Kobori et al., 2018). Thus, the effect of altered physical environments on Mϕ differentiation/activation may consequently dysregulate Mϕ chemical signaling to other tissues.

3 CONCLUSIONS AND RECOMMENDATIONS

In summary, we have discussed the hypothetical multiscale involvement of the MRTF-A/SRF pathway in the dysregulation of Mϕ under microgravity and radiation. MRTF-A is a potential regulator and adaptor of cytoskeletal architecture, migration, phagocytosis, ROS generation, cytokine secretion/expression, and adherence proteins. However, MRTF-A/SRF has many complications; its function is dependent on cell type and is not completely understood in Mϕ. MRTF-A is post-translationally acetylated, phosphorylated, or SUMOylated by many factors, including intracellular crosstalk with other mechanotransducive pathways such as ERK (Panayiotou et al., 2016), YAP/TAZ (Lopez-Hernandez et al., 2021), and p38 MAPK in Mϕ (Ronkina et al., 2016), that alter its cellular localization. Additionally, the nuclear transport of MRTF depends on cytoskeletal/nucleoskeletal architecture. Related mechanical factors such as shear stress and oscillation in simulated microgravity bioreactors may also influence MRTF. For example, emerin-based nucleoskeletal dynamics affect MRTF translocation (Ho et al., 2013). Not only mechanical but also chemical factors, such as hypoxia and oxidative stress, induce the MRTF/SRF pathway in Mϕ (Yang et al., 2020). Therefore, we recommend that future studies attempt to pinpoint MRTF-A/SRF modulation to one of these factors, not excluding microgravity.

We have primarily discussed the connection of MRTF-A to the actin cytoskeleton. However, we also recommend further study in microtubule disruption that may alter the p38 MAPK pathway. While p38 MAPK/MK2 is known to mediate phosphorylation of MRTF-A in Mϕ, the consequence of which is not yet known (Ronkina et al., 2016). Furthermore, the consequence of radiation damage on microtubules is rarely studied although may be negligible (Zaremba and Irwin, 1981; Bruni et al., 2020). It is possible that radiation alters the transport of p38 MAPK and p65 NF-κB on microtubules. Thus, the two separate effects may modulate different pathways: NF-κB may depend on radiation and MRTF may depend on microgravity. To test this, we first recommend co-quantification of the MRTF-A vs. p65 NF-κB nuclear/cytoplasmic ratio, compared with the F/G actin ratio, under simulated microgravity followed by such in simulated radiation.

Mϕ are one of the most radioresistant and redox-resistant cell types, important for their role in the clearance of radiation-damaged, apoptotic cells (Meziani et al., 2018). However, Mϕ are mechano-sensitive and uniquely mechano-regulated as described previously. Importantly, the dominant effects of microgravity vs. radiation depend on cell type, thus directed treatment of spaceflight diseases should be specific to cell type. For example, spaceflight acceleration of atherosclerosis could be treated by activating p53, as it plays a crucial role in preventing the disease (Merched et al., 2003). However, p53 in Mϕ potentiates inflammation and is already upregulated in microgravity (Shi et al., 2021), thus by activating p53 we may inadvertently expedite spaceflight immune dysregulation.

MRTF-A is widely expressed across many cell types and is implicated in cardiovascular, musculoskeletal, and immune diseases (Gau and Roy, 2018) relevant to spaceflight. For instance, MRTF-A is upregulated in blood-circulating Mϕ associated with atherosclerotic lesions, thus treatment that supplants MRTF-A may inadvertently accelerate atherosclerosis in space. Similar conclusions can be made with spaceflight diseases such non-alcoholic fatty liver disease (Beheshti et al., 2019), related to MRTF (Zhang et al., 2021). Currently, no safe drugs have been proven for the treatment of space-induced cardiovascular disease, and evaluations of potential drugs is often contradictory (Meerman et al., 2021). In conclusion, future investigation of treatment for spaceflight diseases can be improved a multiscale mechanobiological understanding of the consequence of microgravity × radiation environments on Mϕ. Our work contributes to this understanding by introducing MRTF.

4 Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5 Author Contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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