

Hypothesis

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

Rocky An^{1,2*}

¹Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA

²Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA

* Correspondence: ra474@cornell.edu

Abstract

Macrophages exhibit impaired phagocytosis, adhesion, migration, and cytokine production in space, hindering their ability to elicit immune responses. Considering that the combined effect of spaceflight microgravity and radiation is multiscale and multifactorial in nature, it is expected that contradictory findings are common in the field. This theory paper reanalyzes research on the macrophage spaceflight response across multiple timescales from seconds to weeks, and spatial scales from the molecular, intracellular, extracellular, to the physiological. Key findings include time-dependence of both pro-inflammatory activation and integrin expression. Here, we introduce the time-dependent, intracellular localization of MRTF-A as a hypothetical confounder of macrophage activation. We discuss the mechanosensitive MRTF-A/SRF pathway dependence on the actin cytoskeleton/nucleoskeleton, microtubules, membrane mechanoreceptors, hypoxia, oxidative stress, and intracellular/extracellular crosstalk. By adopting a multiscale perspective, this paper provides the first mechanistic answer for a three-decade-old question regarding impaired cytokine secretion in microgravity—and strengthens the connection between the recent advances in mechanobiology, microgravity, 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.

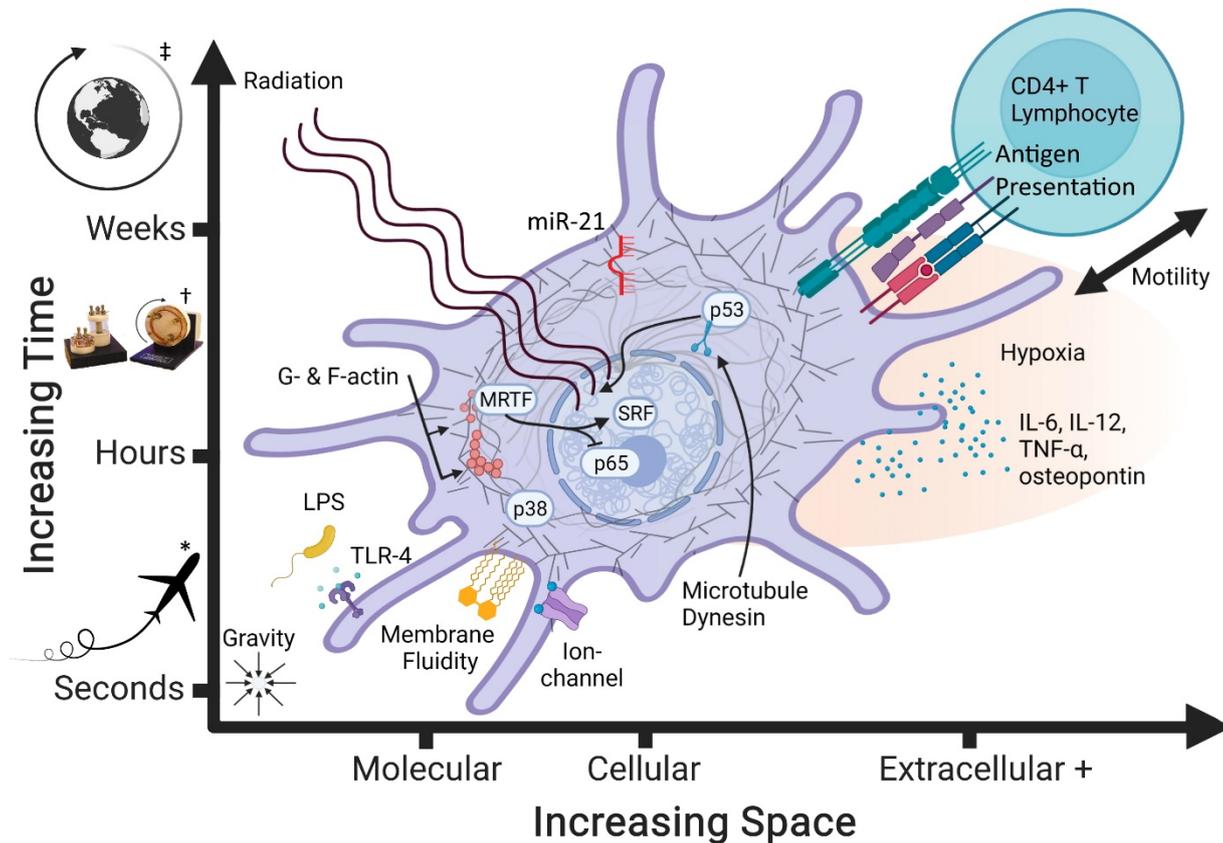
1 INTRODUCTION

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 (Mak et al., 2015; 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 (seconds) to ballistic flights (minutes) to long-term spaceflight (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 ($M\phi$), 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, immune cell-cell interaction, 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, however, mechanistic

understandings are necessary for safe, effective treatment of spaceflight diseases such as immune dysregulation (Crucian 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 M ϕ toward atherosclerotic lesions because of many factors including radiation (Patel, 2020), reactive oxygen species (ROS) (Y. Wang et al., 2014), adhesion proteins (Yang et al., 2005), and motility (Mukherjee et al., 2022)—all of which are afflicted by spaceflight



Figure

1. Simplified model. Some M ϕ spaceflight effects require more time or 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, G-actin—globular actin, F-actin—filamentous actin, microtubules, dynein, p53—tumor protein P53, p38—mitogen-activated protein kinase p38, MRTF—myocardin-related transcription factor A, SRF—serum response factor, NF- κ B/p65—nuclear factor kappa-light-chain-enhancer of activated B cells (p65 or RelA), IL-6—interleukin 6, IL-12, TNF- α —tumor necrosis factor-alpha, osteopontin, miR-21—microRNA-21-5p). 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. *Parabolic Flight, †Simulated microgravity culture vessel, ‡Long-term orbital spaceflight.

Here, we apply a multiscale approach incorporating microgravity, mechanotransduction, radiation, and crosstalk to propose mechanisms for the most well-studied M ϕ 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 the role of emerin—a putative gravi-sensitive nuclear envelope protein (Aventaggiato et al., 2020; Vahlensieck et al., 2022)—, novel microgravity mechanisms for arginase-1 (*ARG1*) regulation, and, most notably, a novel scale in the multiscale space milieu via the myocardin-related transcription factor-A/serum-response factor (MRTF-A/SRF) pathway. In M ϕ , transcriptomic analysis is blind to the intracellular localization of MRTF-A. Furthermore, MRTF-A is currently not included in any KEGG database pathway, and its transcription program may be concealed by overarching pro-inflammatory signaling pathways. Mutations in

MRTF-A cause severe immunodeficiency (Record et al., 2021). Thus, introducing MRTF reinforces space studies that would otherwise have seemingly contradictory conclusions regarding suppression or activation of the pro-inflammatory (classical M1) response of the uniquely mechano-regulated M ϕ cell type.

1.2 MRTF-A transduces M ϕ pro-inflammatory signals

M ϕ pro-inflammatory activation and cytoskeletal reorganization occurs in a biphasic manner (Jain and Vogel, 2018; Ronzier et al., 2022): firstly in a chemical and secondly a mechanotransductive phase lasting 0-3 hours and 3-24 hours respectively. In the first stage, activation of surface receptors induces NF- κ B/p65 nuclear translocation. Secondly, actin polymerization modulates cytokine transcription/secretion via transport of MRTF-A to the nucleus where it slowly accumulates over three hours and associates with serum response factor (SRF) or NF- κ B/p65 transcription factors, or independently binds to SAP motifs of DNA (Olson and Nordheim, 2010; Gau and Roy, 2018; Zhou et al., 2021). The mechanosensitivity of MRTF-A is well-studied; if mechanical force induces polymerization of globular (G)-actin to filamentous (F)-actin, then G-actin-bound MRTF-A is released and translocated to the nucleus (simplified “classical” model):



Figure 2 presents a simplified mechanistic overview of MRTF in M ϕ pro-inflammatory activation. Target genes include interleukin 6 (IL-6), IL-1 β , 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 M ϕ . This is supported with the understanding that M ϕ activation is metabolically regulated by epigenetic “brakes” (Ivashkiv, 2013), and that MRTF physically interacts with NF- κ B/p65 resulting in the mutual inhibition of them both (Miranda et al., 2021) (Figure 2). 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 M ϕ in microgravity.

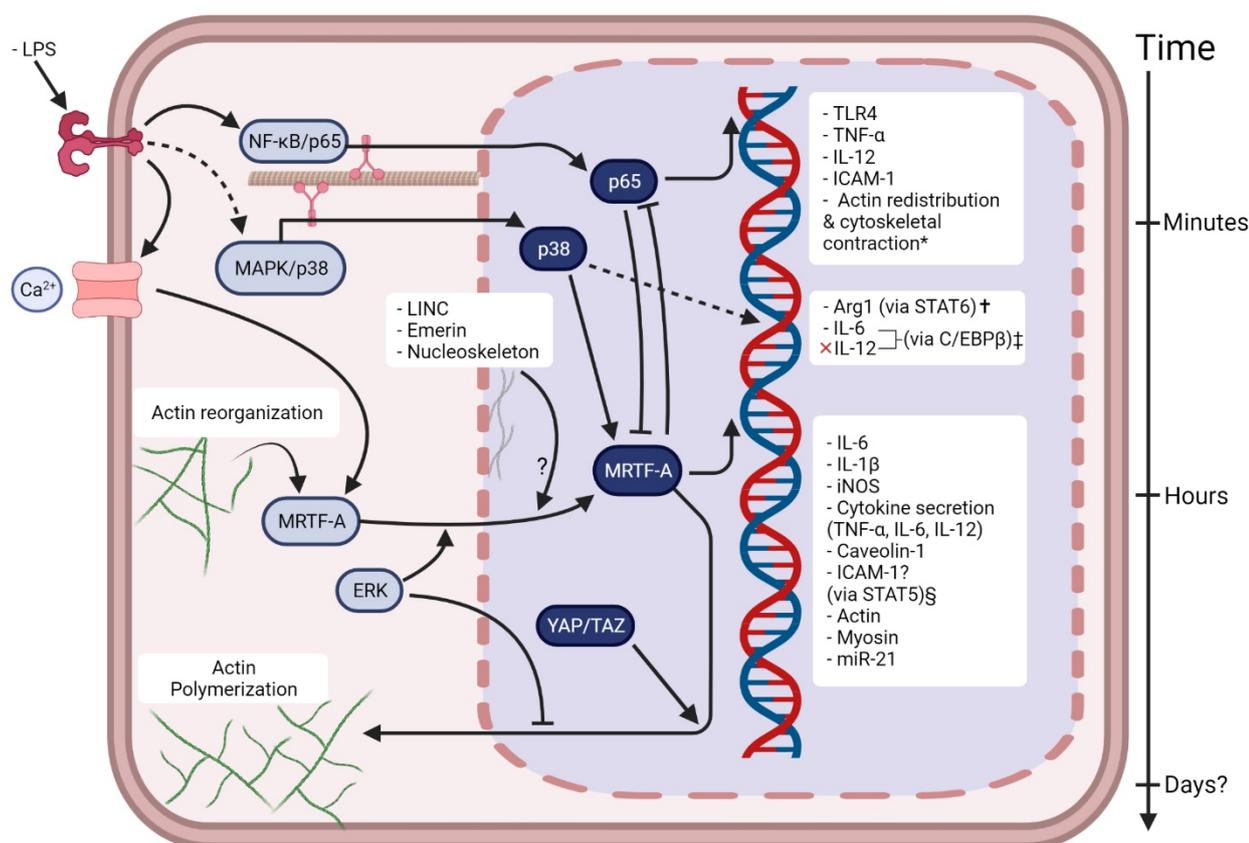


Figure 2. Mechanotransduction is inherently involved in Mφ activation in a time-dependent manner. In controlled conditions, LPS induces TLR4 “outside-in” signaling in the first few hours of activation. Sustained LPS induces “inside-out” signaling, resulting in activation of ion-channels and actin reorganization. This causes a second mechanotransductive phase via the accumulation of MRTF-A. Many pathways are simplified for clarity. The complicated involvement of cellular mechanical mechanisms (LINC—linker of nucleoskeleton and cytoskeleton, emerin, ERK—extracellular signal-regulated kinase, YAP/TAZ—Yes-associated protein/tafazzin), or physical environments (substrate stiffness, spatial confinement, microgravity) alters the extent of Mφ activation. The bidirectional interaction of p65 and MRTF does not fully inhibit them as some late-transcriptional genes are promoted by p65 & MRTF complex i.e., *iNOS* (Miranda et al., 2021). Created with BioRender.com. *(Bian et al., 2017; Mu, 2018; Ronzier et al., 2022), †(Jiménez-García et al., 2015), ‡(C. Wang et al., 2015), §(Miranda et al., 2021).

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? This has been an open question for three decades (Chapes et al., 1992). The microgravity effect on the MRTF-A/SRF pathway has not been explored in Mφ and has been rarely explored in other cell types. Chang et al. (2012) analyzed astronaut T-cell transcriptomic profiles, finding the majority of downregulated genes to be promoted by SRF. Later, in a similar spaceflight study by Hughes-Fulford et al., (2015) it was found that microRNA-21 (miR-21) was downregulated. Relatedly, miR-21 is promoted by MRTF/SRF and is attributed to pro-inflammatory activation in Mφ (Z. Wang et al., 2015; Li et al., 2017). We emphasize the importance of research in Mφ because they are implicated in diseases associated with spaceflight, such as atherosclerosis—where plaque-associated Mφ overexpress MRTF-A (An et al., 2019)—as well 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).

2 MULTISCALE ANALYSIS IN APPROX. INCREASING ORDER OF SPACE AND TIME

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 M ϕ phenotype throughout the body. Innate immune system function necessitates M ϕ 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 M ϕ repolymerizes actin and corrects it within minutes (Thiel et al., 2019). We speculate that feedback loops associated with the cellular level of actin polymerization are involved. For example, MRTF can recruit protein complexes associated with chromatin remodeling (Miranda et al., 2021). The actin nucleoskeleton also regulates and remodels chromatin (Venit et al., 2021), and is similarly restructured in microgravity resulting in the modulation of mechano-sensitive genes (Neelam et al., 2020).

Furthermore, the M ϕ cytoskeleton is physically linked with the cytoplasmic membrane. This linkage mediates motility and phagocytosis (Liu et al., 2020). Less studied in microgravity, there is evidence presented by Kohn et al. (2017) that microgravity increases lipid membrane fluidity or decreases membrane tension. If this is true, then lipid rafts could be disrupted, for instance allowing free diffusion of caveolin-1 (Le Roux et al., 2019)—a crucial protein for M ϕ phagocytosis (Li et al., 2005; Rubio et al., 2018). We mention that the quick response of the plasma membrane to mechanical forces may also play a role in the M ϕ oxidative burst reaction—which rapidly adapts to microgravity (Adrian et al., 2013; Thiel et al., 2017).

Membrane ion channels are also rapidly sensitive to membrane tension/fluidity and are known to have importance to inflammation, for instance inducing MRTF (Sharma et al., 2017). However, ion-channels are rarely studied in microgravity despite their mechanosensitivity (Ludtka and Silberman et al., 2021). The two well-known mechano-sensitive Ca²⁺ 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 ion channel, Hv1, is responsible for inducing superoxide production for the M ϕ oxidative burst reaction after phagocytosis (Ramsey et al., 2009). 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 parabolic flight with a gravity period of ~60 seconds.

2.2 Mechanotransduction

Gene expression is often studied on the timescale of hours in simulated microgravity bioreactors, which oscillate the gravity force usually between 10-15 rpm. Expression is not only induced by biochemical signaling, but also from the direct 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 M ϕ cell). They identified emerin, a ubiquitous nuclear lamin protein, to be involved independently from the nucleoskeleton. We identify emerin to be a potential confounding cause of nuclear stiffness discrepancies across simulated/spaceflight microgravity platforms—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 methods that vary in substrate rigidity, adhesion, or extracellular matrix (ECM). Here, cells cultured on both rigid substrates and at 10-15 R.P.M. (close to 9 /min where emerin nuclear stiffening was observed) are more motile, stiffer, or exhibit greater actin polymerization (Janmaleki et al., 2016; Mao et al., 2016; Thompson et al., 2020; Wubshet et al., 2021), apparently contradicting general findings of spaceflight microgravity studies. Moreover, we mention that

emerin is known to be dependent on substrate-stiffness for modulating MRTF-A levels in the nucleus (Record et al., 2021).

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 M ϕ (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* that is observed in M ϕ in simulated and spaceflight microgravity (C. Wang et al., 2015; Ludtka and Silberman et al., 2021). In fact, M ϕ *ARG1* expression is induced by perturbing microtubules via chemical methods, yet is not affected by chromatin remodeling nor by ECM stiffness (Meizlish, 2021). Alternatively, p38 MAPK induction is linked to mechanosensitive membrane proteins (Cuenda and Rousseau, 2007). The timescale difference between membrane proteins and microtubule arrangement could factor in M ϕ arginine level modulation variation between short- and long-term spaceflight (Thiel et al., 2021).

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. Although in the field of cell adhesion and migration, the generalized effect of cell mechanical characteristics is still unclear (Mierke, 2021). 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 umbilical vein endothelial cells	3D-RPM, ~10 rpm	Petri Dish	↓ Cell stiffness ↓ F-actin, microtubules	(Janmaleki et al., 2016)
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)
Mouse mesenchymal stem cell	RWV, 15 rpm	SlideFlasks (2D plated cells)	↓ Nuclear stiffness (not significant) ↓ F-actin (not significant)	(Thompson et al., 2020)

2.3 Intracellular localization and transport

Upon sustained LPS stimulation, MRTF-A/SRF cytoskeletal mechanotransduction from M ϕ activation is a slow process that takes up to four hours vs. a few minutes for NF- κ B (Bagaev et al., 2019). We hypothesize that delayed mechanotransduction causes 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-1 β is significantly downregulated after 4-24 hours, concordant with our theory that actin disruption in microgravity inhibits the MRTF-A/SRF pathway. Interestingly, if normal gravity is restored post-48 hours, then cytokine expression/secretion appears to recover (Table 2). Likewise, there is no time dependence of NF- κ B-dependent TNF- α expression/secretion as it is consistently downregulated in both simulated and spaceflight microgravity. We also consider an alternative mechanotransductive pathway, p38 MAPK, in two studies where the data are available (Table 2), which may explain inconsistency in Table 2 regarding IL-6 and IL-12 expression/secretion, because p38 MAPK activation results in increased *IL-6* and decreased *IL-12b* expression (C. Wang et al., 2015).

Our identification of MRTF-A/SRF pathway inhibition is the first time that altered M ϕ cytokine profiles have been linked to microgravity. Not only cytokines, but also a previous experiment (Hsieh et al., 2005) (Table 2) showed reduced nitric oxide (NO) secretion. In correlation, MRTF-A/SRF promotes iNOS (Yang et al., 2020) which is essential for killing pathogens after phagocytosis. We also conjecture that MRTF-A is a factor in impaired M ϕ phagocytosis in microgravity. MRTF-A-promoted genes involved in phagocytosis include caveolin-1 (*CAV1*) (Krawczyk et al., 2015) and intercellular adhesion molecule-1 (*ICAM-1*) (Zhong et al., 2021; Huang et al., 2022). Unfortunately, *ICAM-1* regulation by MRTF-A is not consistent across cell type and is unclear in M ϕ , and it may also be NF- κ B-dependent (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 M ϕ , it is apparently time-dependent (Table 3), but no microgravity-linking mechanism has been identified yet.

Table 2. After M ϕ stimulation, cytokine responses are altered under microgravity over time
Boldened results indicate a reduction in pro-inflammatory cytokines TNF- α /IL-6/IL-12/IL-1 β , 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 M ϕ after RWV	RWV, 18 rpm	10-mL RCCS-D bulk vessels (Synthecon)	1, 2, 3 hr after 12 hr differentiation and 72 hr RWV	<p>↓ IL-6 secretion, expression, exacerbated over time</p> <p>↓ TNF-α secretion, exacerbated over time</p> <p>↓ TNF-α expression</p> <p>↓ p38 MAPK pathway</p>	(Wang et al., 2020)
RAW 264.7 & primary mouse M ϕ	RWV, unspecified rpm	Adherent microcarrier beads	4 hr after 24 hr RWV	<p>↓ IL-6, IL-12 secretion</p> <p>↓ TNF-α secretion</p> <p>Unchanged p38 MAPK pathway</p>	(C. Wang et al., 2014)
Primary mouse M ϕ	RWV, 12-25 rpm	Adherent microcarrier beads	4 hr after 24 hr RWV	<p>IL-6 expression and concentration</p> <p>↓ IL-12 subunit B expression</p> <p>p38 MAPK pathway</p>	(C. Wang et al., 2015)
			24 hr after 24 hr RWV	<p>↓ (less significant) IL-12 subunit B concentration</p> <p>p38 MAPK pathway</p>	
RAW 264.7 murine M ϕ	RWV, 14 rpm	10-mL RCCS-D bulk vessels (Synthecon)	48 hr after 48 hr RWV	<p>↓ IL-6, IL-12 secretion</p> <p>↓ TNF-α, NO secretion</p>	(Hsieh et al., 2005)
Human blood monocyte stimulated with LPS	Spaceflight	<i>In vivo</i> , then whole blood cultured, and stimulated	under 1g 48 hr, after ~350 hr spaceflight	<p>↓ IL-6 expression</p> <p>IL-1β expression</p> <p>↓ TNF-α expression</p> <p>↓ IL-10 expression</p>	(Crucian et al., 2011)
Mouse splenocyte stimulated with LPS	Spaceflight	<i>In vivo</i> , then flat-bottom plated, and stimulated	under 1g 48 hr, after ~312 hr spaceflight	<p>IL-6 secretion</p> <p>IL-12 (ns)</p> <p>↓ TNF-α secretion</p> <p>IL-10 secretion</p>	(Baqai et al., 2009)
RAW 264.7 murine M ϕ	RWV, 14 rpm	Adherent microcarrier beads	72 hr RWV after 48 hr of stimulation	<p>IL-6 (ns)</p> <p>IL-12 secretion</p> <p>↓ TNF-α secretion</p> <p>IL-10 secretion</p>	(Ludtka and Moore et al., 2021)

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). Induction of M ϕ ICAM-1 levels off after ~12 hours (according to Zhong et al., (2021) with 0 hr, 12 hr, and 24 hr time points). Therefore, we postulate that MRTF-A is a delayed regulator of ICAM-1 expression in M ϕ . In a similar mechanism, Hayashi et al. (2015) found that in vascular endothelial cells, nuclear MRTF-

A binds to NF- κ B/p65, inhibiting p65 promotion of *ICAM-1*. The involvement of both NF- κ B 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 M ϕ to non-differentiated monocytes, a cell type that exhibits unchanged *ICAM-1* levels during microgravity flights. Correspondingly, microarray analysis of these monocytes has shown that only two pathways are weakly altered after six minutes of pro-inflammatory stimulation: NF- κ B, 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 phase of pro-inflammatory activation. Comparatively in M ϕ and pre-differentiated monocytes, relative surface *ICAM-1* levels trended downwards with time (Table 3). We interpret this as either as a resurgence of MRTF-A as the actin cytoskeleton recovers after 24 hours or as a separate, unknown mechanism for *ICAM-1* downregulation in the long term. For example, microRNA-21 is downregulated in T-cells under microgravity (Hughes-Fulford et al., 2015). miR-21 is MRTF/SRF promoted, and attributed to “mechanical memory” of at least 20 days in mesenchymal stem cell fibrogenesis (Z. Wang et al., 2015; Li et al., 2017). Relatedly in M ϕ , miR-21 increases expression of *ICAM-1* (Lu et al., 2020).

Table 3. ICAM-1 surface expression over time in differentiated but non-differentiated M ϕ /monocytes. Simulated and spaceflight microgravity modulated U937 and human M ϕ 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-translational regulatory factors.

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

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, M ϕ 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 M ϕ 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 M ϕ hypoxia in culture. Overall effects may include activation of the p38 MAPK pathway (Paardekooper et al., 2018; Ke et al., 2019)—a pathway that exhibits contradictory activation or suppression in simulated microgravity (Table 2). Another potential

effect is altered metabolism, as glycolytic lactic acid accumulation in culture may stimulate pro-inflammatory cytokine expression in M ϕ (Shi et al., 2021). Whether the microgravity altering effect on M ϕ metabolism can be attributed to both mechanical factors and hypoxic state remains to be elucidated.

Based on the paucity of evidence linking hypoxia with mechanotransduction, it is most likely there is only indirect interaction between the two. Independent of hypoxia, inflammatory cytokines such as IL-6, IL-18, and TNF- α induce hypoxia-inducible factors (HIF) in M ϕ (Vogel et al., 2019). HIF-1 α is well studied in microgravity: Ludtka and Moore et al. (2021) cultured M ϕ on adherent microbeads in RWV and observed no significant change of *HIF-1 α* expression in M ϕ , yet observed upregulation of vascular endothelial growth factor (VEGF) secretion and downregulation of *TNF- α* . It is unclear whether this finding is caused by hypoxia, ROS, or mechanotransductive 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-1 α dependent inhibition of RhoA, a key remodeler of the actin cytoskeleton, overall hindering the MRTF/SRF pathway (Leinhos et al., 2019). Furthermore, hypoxia upregulates M ϕ expression of ICAM-1 likely in a p53 or NF- κ B dependent manner (Gorgoulis et al., 2003). Generally, hypoxia polarizes M ϕ toward anti-inflammatory phenotypes (Ke et al., 2019). Thus, we hypothesize that hypoxia and microgravity act independently to suppress M ϕ pro-inflammatory phenotype.

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 M ϕ 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 (alternative M2) activation—possibly by inactivation of p38 MAPK—but high doses induce pro-inflammatory (classical M1) activation, possibly by activation of p53—a well-studied transcription factor that stimulates DNA repair or apoptosis. Alternatively, p53 is transported by dynein on microtubules (Giannakakou et al., 2000), similar to p38 MAPK (see section 2.2. Mechanotransduction).

The abrogation of M ϕ phenotypic disorder observed in space may be misattributed to adaptation to microgravity instead of the long-term effects of radiation. For instance, we hypothesize the apparent reversal of ARG1 (Thiel et al., 2021) and surface ICAM-1 expression between 11-30 days in orbital spaceflight (Table 3) to be caused by inactivation of either p38 MAPK or downregulation of miR-21 (see section 2.3). 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 (see section 2.1). 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- κ B) respectively; both transcription factors form a complex to promote *iNOS* (Miranda et al., 2021) and ROS-producing *NOX4* (Liu et al., 2018). 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). Therefore, chronic ROS generation could be another mechanism for the apparent reversal of ICAM-1 surface expression in spaceflight.

2.6 Intercellular and physiological crosstalk

M ϕ dysregulation translates to impaired interaction with other immune cells. For example, T lymphocyte interaction is essential for antigen presentation, but may be slowed by M ϕ migration impairment in microgravity (Meloni et al., 2006). Additionally, M ϕ reduced surface ICAM-1 expression in spaceflight (Table 3) may hinder their adhesion and subsequent activation of CD4⁺ T lymphocytes (Lin et al., 2020). Not only considering immune cells, 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 M ϕ . Here, p38 and ERK was rescued by probiotics, thus microgravity may

mechanically regulate the microbiota-immune axis. Lastly, M ϕ are mediators of intercellular signals. As observed in coculture by Fu et al. (2019), radiation-induced apoptosis signaling is propagated by M ϕ , potentially increasing tissue damage. Damaged-cell intercellular signaling is enough to stimulate M ϕ differentiation/activation, regardless of M ϕ irradiation state.

Monocyte/M ϕ differentiation also depends on both microgravity and radiation. Shi et al. (2021) observed that microgravity suppresses differentiation of M ϕ to either pro-inflammatory or anti-inflammatory phenotype; yet, Coates et al (2008) observed that radiation augments M ϕ differentiation. Earlier (section 2.5), we have hypothesized that—regarding the innate immune response—radiation counteracts microgravity. But regarding bone degeneration, the effect of microgravity and radiation appears additive by increased fusion of monocyte/M ϕ in forming multinucleated osteoclasts (Shanmugarajan et al., 2017). Osteal M ϕ 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 M ϕ (Fantuzzi, 2003) generally promoting phagocytic activity (Schuch et al., 2016). M ϕ produces osteopontin when stimulated with anti-inflammatory IL-18 and IL-10 (Kobori et al., 2018), both of which are regulated by oxidative and mechanical stress. Thus, the effect of altered physical environments on M ϕ differentiation/activation may consequently dysregulate M ϕ 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 M ϕ 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 M ϕ . MRTF-A is post-translationally acetylated, phosphorylated, or SUMOylated by many factors, including intracellular crosstalk with other mechanotransductive pathways such as ERK (Panayiotou et al., 2016), YAP/TAZ (Lopez-Hernandez et al., 2021), and p38 MAPK in M ϕ (Ronkina et al., 2016), that alter its cellular localization. Additionally, the nuclear transport of MRTF depends on nuclear lamina-associated proteins as well as cytoskeletal/nucleoskeletal architecture (Ho et al., 2013; Sidorenko et al., 2022). Related mechanical factors such as shear stress, vibration, and oscillation in simulated microgravity bioreactors may also influence MRTF translocation. Not only mechanical but also chemical factors, such as hypoxia and oxidative stress, induce the MRTF/SRF pathway in M ϕ (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 M ϕ , 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- κ B on microtubules. Thus, the two separate effects may modulate different pathways: NF- κ B 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- κ B nuclear/cytoplasmic ratio, compared with the F/G actin ratio, under simulated microgravity followed by such in simulated radiation.

M ϕ 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, M ϕ 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 M ϕ 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 M ϕ associated with atherosclerotic lesions, thus a drug 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 \times radiation environments on M ϕ . 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.

6 References

- Adrian, A., Schoppmann, K., Sromicki, J., Brungs, S., von der Wiesche, M., Hock, B., et al. (2013) ‘The oxidative burst reaction in mammalian cells depends on gravity’, *Cell Communication and Signaling*, 11(1), p. 98. doi:10.1186/1478-811X-11-98.
- An, J., Naruse, T.K., Hinohara, K., Soejima, Y., Sawabe, M., Nakagawa, Y., et al. (2019) ‘MRTF-A regulates proliferation and survival properties of pro-atherogenic macrophages’, *Journal of Molecular and Cellular Cardiology*, 133, pp. 26–35. doi:10.1016/j.yjmcc.2019.05.015.
- An, R. and Lee, J.A. (2022) ‘CAMDLES: CFD-DEM Simulation of Microbial Communities in Spaceflight and Artificial Microgravity’, *Life*, 12(5), p. 660. doi:10.3390/life12050660.
- Aventaggiato, M., Barreca, F., Vernucci, E., Bizzarri, M., Ferretti, E., Russo, M.A., et al. (2020) ‘Putative Receptors for Gravity Sensing in Mammalian Cells: The Effects of Microgravity’, *Applied Sciences*, 10(6), p. 2028. doi:10.3390/app10062028.
- Bagaev, A.V., Garaeva, A.Y., Lebedeva, E.S., Pichugin, A.V., Ataulakhanov, R.I. and Ataulakhanov, F.I. (2019) ‘Elevated pre-activation basal level of nuclear NF- κ B in native macrophages accelerates LPS-induced translocation of cytosolic NF- κ B into the cell nucleus’, *Scientific Reports*, 9, p. 4563. doi:10.1038/s41598-018-36052-5.
- Baqai, F.P., Gridley, D.S., Slater, J.M., Luo-Owen, X., Stodieck, L.S., Ferguson, V., et al. (2009) ‘Effects of spaceflight on innate immune function and antioxidant gene expression’, *Journal of Applied Physiology*, 106(6), pp. 1935–1942. doi:10.1152/jappphysiol.91361.2008.
- Basirun, C., Ferlazzo, M.L., Howell, N.R., Liu, G.-J., Middleton, R.J., Martinac, B., et al. (2021) ‘Microgravity \times Radiation: A Space Mechanobiology Approach Toward Cardiovascular Function and Disease’, *Frontiers in Cell and Developmental Biology*, 9, p. 3079. doi:10.3389/fcell.2021.750775.
- Beheshti, A., Chakravarty, K., Fogle, H., Fazelinia, H., Silveira, W.A. da, Boyko, V., et al. (2019) ‘Multi-omics analysis of multiple missions to space reveal a theme of lipid dysregulation in mouse liver’, *Scientific Reports*, 9(1), p. 19195. doi:10.1038/s41598-019-55869-2.
- Bhattacharya, S., Calar, K. and de la Puente, P. (2020) ‘Mimicking tumor hypoxia and tumor-immune interactions employing three-dimensional in vitro models’, *Journal of Experimental & Clinical Cancer Research*, 39(1), p. 75. doi:10.1186/s13046-020-01583-1.
- Bian, H., Li, F., Wang, W., Zhao, Q., Gao, S., Ma, J., et al. (2017) ‘MAPK/p38 regulation of cytoskeleton rearrangement accelerates induction of macrophage activation by TLR4, but not TLR3’, *International Journal of Molecular Medicine*, 40(5), pp. 1495–1503. doi:10.3892/ijmm.2017.3143.
- Botello-Smith, W.M., Jiang, W., Zhang, H., Ozkan, A.D., Lin, Y.-C., Pham, C.N., et al. (2019) ‘A mechanism for the activation of the mechanosensitive Piezo1 channel by the small molecule Yoda1’, *Nature Communications*, 10(1), p. 4503. doi:10.1038/s41467-019-12501-1.
- Brungs, S., Hauslage, J. and Hemmersbach, R. (2019) ‘Validation of Random Positioning Versus Clinorotation Using a Macrophage Model System’, *Microgravity Science and Technology*, 31(2), pp. 223–230. doi:10.1007/s12217-019-9687-0.

- Bryant, S.L., Shrestha, N., Oxford, J., Cornell, K. and Fologea, D. (2017) 'Simulated Microgravity Conditions Modulate Ca²⁺ Transport through TRPV4 Channels', *Biophysical Journal*, 112(3), p. 251a. doi:10.1016/j.bpj.2016.11.1371.
- Buravkova, L.B., Rudimov, E.G., Andreeva, E.R. and Grigoriev, A.I. (2018) 'The ICAM-1 expression level determines the susceptibility of human endothelial cells to simulated microgravity', *Journal of Cellular Biochemistry*, 119(3), pp. 2875–2885. doi:10.1002/jcb.26465.
- Cess, C.G. and Finley, S.D. (2020) 'Multi-scale modeling of macrophage-T cell interactions within the tumor microenvironment', *PLoS computational biology*, 16(12), p. e1008519. doi:10.1371/journal.pcbi.1008519.
- Chang, T.T., Walther, I., Li, C.-F., Boonyaratanakornkit, J., Galleri, G., Meloni, M.A., et al. (2012) 'The Rel/NF- κ B pathway and transcription of immediate early genes in T cell activation are inhibited by microgravity', *Journal of Leukocyte Biology*, 92(6), pp. 1133–1145. doi:10.1189/jlb.0312157.
- Chapes, S.K., Morrison, D.R., Guikema, J.A., Lewis, M.L. and Spooner, B.S. (1992) 'Cytokine secretion by immune cells in space', *Journal of Leukocyte Biology*, 52(1), pp. 104–110. doi:10.1002/jlb.52.1.104.
- Crucian, B., Stowe, R., Quiariarte, H., Pierson, D. and Sams, C. (2011) 'Monocyte phenotype and cytokine production profiles are dysregulated by short-duration spaceflight', *Aviation, Space, and Environmental Medicine*, 82(9), pp. 857–862. doi:10.3357/asem.3047.2011.
- Crucian, B.E., Choukèr, A., Simpson, R.J., Mehta, S., Marshall, G., Smith, S.M., et al. (2018) 'Immune System Dysregulation During Spaceflight: Potential Countermeasures for Deep Space Exploration Missions', *Frontiers in Immunology*, 9. Available at: <https://www.frontiersin.org/article/10.3389/fimmu.2018.01437> (Accessed: 28 May 2022).
- Cuenda, A. and Rousseau, S. (2007) 'p38 MAP-Kinases pathway regulation, function and role in human diseases', *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1773(8), pp. 1358–1375. doi:10.1016/j.bbamcr.2007.03.010.
- Decano, J.L. and Aikawa, M. (2018) 'Dynamic Macrophages: Understanding Mechanisms of Activation as Guide to Therapy for Atherosclerotic Vascular Disease', *Frontiers in Cardiovascular Medicine*, 5. Available at: <https://www.frontiersin.org/article/10.3389/fcvm.2018.00097> (Accessed: 28 May 2022).
- Deymier, A.C., Schwartz, A.G., Lim, C., Wingender, B., Kotiya, A., Shen, H., et al. (2020) 'Multiscale effects of spaceflight on murine tendon and bone', *Bone*, 131, p. 115152. doi:10.1016/j.bone.2019.115152.
- ElGindi, M., Sapudom, J., Ibrahim, I.H., Al-Sayegh, M., Chen, W., Garcia-Sabaté, A., et al. (2021) 'May the Force Be with You (Or Not): The Immune System under Microgravity', *Cells*, 10(8), p. 1941. doi:10.3390/cells10081941.
- Fang, F., Yang, Y., Yuan, Z., Gao, Y., Zhou, J., Chen, Q., et al. (2011) 'Myocardin-Related Transcription Factor A Mediates OxLDL-Induced Endothelial Injury', *Circulation Research*, 108(7), pp. 797–807. doi:10.1161/CIRCRESAHA.111.240655.
- Fantuzzi, G. (ed.) (2003) *Cytokine Knockouts*. Totowa, NJ: Humana Press. doi:10.1007/978-1-59259-405-4.
- Finch-Edmondson, M. and Sudol, M. (2016) 'Framework to function: mechanosensitive regulators of gene transcription', *Cellular & Molecular Biology Letters*, 21(1), p. 28. doi:10.1186/s11658-016-0028-7.
- Fritzsche, M. (2020) 'Thinking multi-scale to advance mechanobiology', *Communications Biology*, 3(1), pp. 1–2. doi:10.1038/s42003-020-01197-5.
- Fu, J., Zhu, L., Tu, W., Wang, X., Pan, Y., Bai, Y., et al. (2019) 'Macrophage-Mediated Bystander Effects after Different Irradiations through a p53-dependent Pathway', *Radiation Research*, 193(2), pp. 119–129. doi:10.1667/RR15354.1.
- Gau, D. and Roy, P. (2018) 'SRF'ing and SAP'ing – the role of MRTF proteins in cell migration', *Journal of Cell Science*, 131(19), p. jcs218222. doi:10.1242/jcs.218222.
- Giannakakou, P., Sackett, D.L., Ward, Y., Webster, K.R., Blagosklonny, M.V. and Fojo, T. (2000) 'p53 is associated with cellular microtubules and is transported to the nucleus by dynein', *Nature Cell Biology*, 2(10), pp. 709–717. doi:10.1038/35036335.
- Gomes, T., Song, Y., Brede, D.A., Xie, L., Gutzkow, K.B., Salbu, B., et al. (2018) 'Gamma radiation induces dose-dependent oxidative stress and transcriptional alterations in the freshwater crustacean *Daphnia magna*', *The Science of the Total Environment*, 628–629, pp. 206–216. doi:10.1016/j.scitotenv.2018.02.039.

- Gorgoulis, V.G., Zacharatos, P., Kotsinas, A., Kletsas, D., Mariatos, G., Zoumpourlis, V., et al. (2003) 'p53 activates ICAM-1 (CD54) expression in an NF- κ B-independent manner', *The EMBO Journal*, 22(7), pp. 1567–1578. doi:10.1093/emboj/cdg157.
- Guenther, C., Faisal, I., Uotila, L.M., Asens, M.L., Harjunpää, H., Savinko, T., et al. (2019) 'A β 2-Integrin/MRTF-A/SRF Pathway Regulates Dendritic Cell Gene Expression, Adhesion, and Traction Force Generation', *Frontiers in Immunology*, 10, p. 1138. doi:10.3389/fimmu.2019.01138.
- Guilluy, C., Osborne, L.D., Van Landeghem, L., Sharek, L., Superfine, R., Garcia-Mata, R., et al. (2014) 'Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus', *Nature Cell Biology*, 16(4), pp. 376–381. doi:10.1038/ncb2927.
- Guo, Y.-J., Pan, W.-W., Liu, S.-B., Shen, Z.-F., Xu, Y. and Hu, L.-L. (2020) 'ERK/MAPK signalling pathway and tumorigenesis (Review)', *Experimental and Therapeutic Medicine*, 19(3), pp. 1997–2007. doi:10.3892/etm.2020.8454.
- de la Haba, C., Palacio, J.R., Martínez, P. and Morros, A. (2013) 'Effect of oxidative stress on plasma membrane fluidity of THP-1 induced macrophages', *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1828(2), pp. 357–364. doi:10.1016/j.bbamem.2012.08.013.
- Hammond, T.G. and Hammond, J.M. (2001) 'Optimized suspension culture: the rotating-wall vessel', *American Journal of Physiology-Renal Physiology*, 281(1), pp. F12–F25. doi:10.1152/ajprenal.2001.281.1.F12.
- Hayashi, K., Murai, T., Oikawa, H., Masuda, T., Kimura, K., Muehlich, S., et al. (2015) 'A novel inhibitory mechanism of MRTF-A/B on the ICAM-1 gene expression in vascular endothelial cells', *Scientific Reports*, 5(1), p. 10627. doi:10.1038/srep10627.
- Ho, C.Y., Jaalouk, D.E., Vartiainen, M.K. and Lammerding, J. (2013) 'Lamin A/C and emerin regulate MKL1–SRF activity by modulating actin dynamics', *Nature*, 497(7450), pp. 507–511. doi:10.1038/nature12105.
- Hsieh, C.-L., Chao, P.-D.L. and Fang, S.-H. (2005) 'Morin sulphates/glucuronides enhance macrophage function in microgravity culture system', *European Journal of Clinical Investigation*, 35(9), pp. 591–596. doi:10.1111/j.1365-2362.2005.01551.x.
- Huang, S., Shao, T., Liu, H., Wang, Q., Li, T. and Zhao, Q. (2022) 'SIRT6 mediates MRTF-A deacetylation in vascular endothelial cells to antagonize oxLDL-induced ICAM-1 transcription', *Cell Death Discovery*, 8(1), pp. 1–10. doi:10.1038/s41420-022-00903-y.
- Hughes-Fulford, M., Chang, T.T., Martinez, E.M. and Li, C.-F. (2015) 'Spaceflight alters expression of microRNA during T-cell activation', *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 29(12), pp. 4893–4900. doi:10.1096/fj.15-277392.
- Ivashkiv, L.B. (2013) 'Epigenetic regulation of macrophage polarization and function', *Trends in Immunology*, 34(5), pp. 216–223. doi:10.1016/j.it.2012.11.001.
- Jaalouk, D.E. and Lammerding, J. (2009) 'Mechanotransduction gone awry', *Nature reviews Molecular cell biology*, 10(1), pp. 63–73.
- Jain, N., Moeller, J. and Vogel, V. (2019) 'Mechanobiology of Macrophages: How Physical Factors Coregulate Macrophage Plasticity and Phagocytosis', *Annual Review of Biomedical Engineering*, 21(1), pp. 267–297. doi:10.1146/annurev-bioeng-062117-121224.
- Jain, N. and Vogel, V. (2018) 'Spatial confinement downsizes the inflammatory response of macrophages', *Nature Materials*, 17(12), pp. 1134–1144. doi:10.1038/s41563-018-0190-6.
- Janmaleki, M., Pachenari, M., Seyedpour, S.M., Shahghadami, R. and Sanati-Nezhad, A. (2016) 'Impact of Simulated Microgravity on Cytoskeleton and Viscoelastic Properties of Endothelial Cell', *Scientific Reports*, 6(1), p. 32418. doi:10.1038/srep32418.
- Jiménez-García, L., Herránz, S., Luque, A. and Hortelano, S. (2015) 'Critical role of p38 MAPK in IL-4-induced alternative activation of peritoneal macrophages', *European Journal of Immunology*, 45(1), pp. 273–286. doi:10.1002/eji.201444806.

- Ke, X., Chen, C., Song, Y., Cai, Q., Li, J., Tang, Y., et al. (2019) 'Hypoxia modifies the polarization of macrophages and their inflammatory microenvironment, and inhibits malignant behavior in cancer cells', *Oncology Letters*, 18(6), p. 5871. doi:10.3892/ol.2019.10956.
- Kim, H., Shin, Y. and Kim, D.-H. (2021) 'Mechanobiological Implications of Cancer Progression in Space', *Frontiers in Cell and Developmental Biology*, 9. Available at: <https://www.frontiersin.org/article/10.3389/fcell.2021.740009> (Accessed: 28 May 2022).
- Kim, J.H., Studer, R.K., Vo, N.V., Sowa, G.A. and Kang, J.D. (2009) 'p38 MAPK inhibition selectively mitigates inflammatory mediators and VEGF production in AF cells co-cultured with activated macrophage-like THP-1 cells', *Osteoarthritis and Cartilage*, 12(17), pp. 1662–1669. doi:10.1016/j.joca.2009.06.004.
- Kitchen, G.B., Cunningham, P.S., Poolman, T.M., Iqbal, M., Maidstone, R., Baxter, M., et al. (2020) 'The clock gene *Bmal1* inhibits macrophage motility, phagocytosis, and impairs defense against pneumonia', *Proceedings of the National Academy of Sciences of the United States of America*, 117(3), pp. 1543–1551. doi:10.1073/pnas.1915932117.
- Kobori, T., Hamasaki, S., Kitaura, A., Yamazaki, Y., Nishinaka, T., Niwa, A., et al. (2018) 'Interleukin-18 Amplifies Macrophage Polarization and Morphological Alteration, Leading to Excessive Angiogenesis', *Frontiers in Immunology*, 9. Available at: <https://www.frontiersin.org/article/10.3389/fimmu.2018.00334> (Accessed: 14 January 2022).
- Kohn, F., Hauslage, J. and Hanke, W. (2017) 'Membrane Fluidity Changes, A Basic Mechanism of Interaction of Gravity with Cells?', *Microgravity Science and Technology*, 29(5), pp. 337–342. doi:10.1007/s12217-017-9552-y.
- Krawczyk, K.K., Yao Mattisson, I., Ekman, M., Oskolkov, N., Granting, R., Kotowska, D., et al. (2015) 'Myocardin Family Members Drive Formation of Caveolae', *PLoS ONE*, 10(8), p. e0133931. doi:10.1371/journal.pone.0133931.
- Krizaj, D., Toft-Bertelsen, T.L., gorusupudi, A., Macaulay, N., Bernstein, P.S. and Lakk, M. (2020) 'Cholesterol regulates TRPV4-dependent signaling in the trabecular meshwork', *Investigative Ophthalmology & Visual Science*, 61(7), p. 3424.
- Le Roux, A.-L., Quiroga, X., Walani, N., Arroyo, M. and Roca-Cusachs, P. (2019) 'The plasma membrane as a mechanochemical transducer', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1779), p. 20180221. doi:10.1098/rstb.2018.0221.
- Leinhos, L., Peters, J., Krull, S., Helbig, L., Vogler, M., Levay, M., et al. (2019) 'Hypoxia suppresses myofibroblast differentiation by changing RhoA activity', *Journal of Cell Science*, 132(5), p. jcs223230. doi:10.1242/jcs.223230.
- Li, C.X., Talele, N.P., Boo, S., Koehler, A., Knee-Walden, E., Balestrini, J.L., et al. (2017) 'MicroRNA-21 preserves the fibrotic mechanical memory of mesenchymal stem cells', *Nature Materials*, 16(3), pp. 379–389. doi:10.1038/nmat4780.
- Li, J., Scherl, A., Medina, F., Frank, P.G., Kitsis, R.N., Tanowitz, H.B., et al. (2005) 'Impaired phagocytosis in caveolin-1 deficient macrophages', *Cell Cycle (Georgetown, Tex.)*, 4(11), pp. 1599–1607. doi:10.4161/cc.4.11.2117.
- Lin, X., Zhang, K., Wei, D., Tian, Y., Gao, Y., Chen, Z., et al. (2020) 'The Impact of Spaceflight and Simulated Microgravity on Cell Adhesion', *International Journal of Molecular Sciences*, 21(9), p. 3031. doi:10.3390/ijms21093031.
- Liu, H., Zhu, L., Dudiki, T., Gabanic, B., Good, L., Podrez, E.A., et al. (2020) 'Macrophage Migration and Phagocytosis Are Controlled by Kindlin-3's Link to the Cytoskeleton', *The Journal of Immunology*, 204(7), pp. 1954–1967. doi:10.4049/jimmunol.1901134.
- Liu, L., Luo, Q., Sun, J. and Song, G. (2019) 'Cytoskeletal control of nuclear morphology and stiffness are required for OPN-induced bone-marrow-derived mesenchymal stem cell migration', *Biochemistry and Cell Biology*, 97(4), pp. 463–470. doi:10.1139/bcb-2018-0263.
- Liu, L., Wu, X., Xu, H., Yu, L., Zhang, X., Li, L., et al. (2018) 'Myocardin-related transcription factor A (MRTF-A) contributes to acute kidney injury by regulating macrophage ROS production', *Biochimica Et Biophysica Acta. Molecular Basis of Disease*, 1864(10), pp. 3109–3121. doi:10.1016/j.bbadis.2018.05.026.

- Lopez-Hernandez, A., Sberna, S. and Campaner, S. (2021) 'Emerging Principles in the Transcriptional Control by YAP and TAZ', *Cancers*, 13(16), p. 4242. doi:10.3390/cancers13164242.
- Lu, X., Yu, Y. and Tan, S. (2020) 'The role of the miR-21-5p-mediated inflammatory pathway in ulcerative colitis', *Experimental and Therapeutic Medicine*, 19(2), pp. 981–989. doi:10.3892/etm.2019.8277.
- Ludtka, C., Moore, E. and Allen, J.B. (2021) 'The Effects of Simulated Microgravity on Macrophage Phenotype', *Biomedicines*, 9(9), p. 1205. doi:10.3390/biomedicines9091205.
- Ludtka, C., Silberman, J., Moore, E. and Allen, J.B. (2021) 'Macrophages in microgravity: the impact of space on immune cells', *npj Microgravity*, 7(1), pp. 1–10. doi:10.1038/s41526-021-00141-z.
- Mak, M., Kim, T., Zaman, M.H. and Kamm, R.D. (2015) 'Multiscale mechanobiology: computational models for integrating molecules to multicellular systems', *Integrative biology: quantitative biosciences from nano to macro*, 7(10), pp. 1093–1108. doi:10.1039/c5ib00043b.
- Mao, X., Chen, Z., Luo, Q., Zhang, B. and Song, G. (2016) 'Simulated microgravity inhibits the migration of mesenchymal stem cells by remodeling actin cytoskeleton and increasing cell stiffness', *Cytotechnology*, 68(6), pp. 2235–2243. doi:10.1007/s10616-016-0007-x.
- McGregor, A.L., Hsia, C.-R. and Lammerding, J. (2016) 'Squish and squeeze – the nucleus as a physical barrier during migration in confining environments', *Current opinion in cell biology*, 40, pp. 32–40. doi:10.1016/j.ceb.2016.01.011.
- Meerman, M., Bracco Gartner, T.C.L., Buikema, J.W., Wu, S.M., Siddiqi, S., Bouten, C.V.C., et al. (2021) 'Myocardial Disease and Long-Distance Space Travel: Solving the Radiation Problem', *Frontiers in Cardiovascular Medicine*, 8. Available at: <https://www.frontiersin.org/article/10.3389/fcvm.2021.631985> (Accessed: 28 May 2022).
- Meizlish, M.L. (2021) *Macrophage Mechanosensing of the Tissue Environment and Signal Integration through the Cytoskeleton*. Ph.D. Yale University. Available at: <https://www.proquest.com/docview/2557411531/abstract/4AF34E8444E0469APQ/1> (Accessed: 25 September 2021).
- Meloni, M.A., Galleri, G., Pani, G., Saba, A., Pippia, P. and Cogoli-Greuter, M. (2011) 'Space flight affects motility and cytoskeletal structures in human monocyte cell line J-111', *Cytoskeleton*, 68(2), pp. 125–137. doi:10.1002/cm.20499.
- Meloni, M.A., Galleri, G., Pippia, P. and Cogoli-Greuter, M. (2006) 'Cytoskeleton changes and impaired motility of monocytes at modelled low gravity', *Protoplasma*, 229(2–4), pp. 243–249. doi:10.1007/s00709-006-0210-2.
- Merched, A.J., Williams, E. and Chan, L. (2003) 'Macrophage-Specific p53 Expression Plays a Crucial Role in Atherosclerosis Development and Plaque Remodeling', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 23(9), pp. 1608–1614. doi:10.1161/01.ATV.0000084825.88022.53.
- Meziani, L., Deutsch, E. and Mondini, M. (2018) 'Macrophages in radiation injury: a new therapeutic target', *Oncoimmunology*, 7(10), p. e1494488. doi:10.1080/2162402X.2018.1494488.
- Mierke, C.T. (2021) 'The Pertinent Role of Cell and Matrix Mechanics in Cell Adhesion and Migration', *Frontiers in Cell and Developmental Biology*, 9. Available at: <https://www.frontiersin.org/articles/10.3389/fcell.2021.720494> (Accessed: 17 July 2022).
- Miranda, M.Z., Lichner, Z., Szász, K. and Kapus, A. (2021) 'MRTF: Basic Biology and Role in Kidney Disease', *International Journal of Molecular Sciences*, 22(11), p. 6040. doi:10.3390/ijms22116040.
- Morrison, T.E. and Kenney, S.C. (2004) 'BZLF1, an Epstein-Barr virus immediate-early protein, induces p65 nuclear translocation while inhibiting p65 transcriptional function', *Virology*, 328(2), pp. 219–232. doi:10.1016/j.virol.2004.07.020.
- Mu, J. (2018) 'RhoA signaling in CCL2-induced macrophage polarization', *Journal of Allergy and Clinical Immunology*, 141(2), p. AB114. doi:10.1016/j.jaci.2017.12.363.
- Mukherjee, P., Rahaman, S.G., Goswami, R., Dutta, B., Mahanty, M. and Rahaman, S.O. (2022) 'Role of mechanosensitive channels/receptors in atherosclerosis', *American Journal of Physiology-Cell Physiology*, 322(5), pp. C927–C938. doi:10.1152/ajpcell.00396.2021.

- Nabavi, N., Khandani, A., Camirand, A. and Harrison, R.E. (2011) 'Effects of microgravity on osteoclast bone resorption and osteoblast cytoskeletal organization and adhesion', *Bone*, 49(5), pp. 965–974. doi:10.1016/j.bone.2011.07.036.
- Neelam, S., Richardson, B., Barker, R., Udave, C., Gilroy, S., Cameron, M.J., et al. (2020) 'Changes in Nuclear Shape and Gene Expression in Response to Simulated Microgravity Are LINC Complex-Dependent', *International Journal of Molecular Sciences*, 21(18), p. 6762. doi:10.3390/ijms21186762.
- Olson, E.N. and Nordheim, A. (2010) 'Linking actin dynamics and gene transcription to drive cellular motile functions', *Nature reviews. Molecular cell biology*, 11(5), pp. 353–365. doi:10.1038/nrm2890.
- Orsini, E.M., Perelas, A., Southern, B.D., Grove, L.M., Olman, M.A. and Scheraga, R.G. (2021) 'Stretching the Function of Innate Immune Cells', *Frontiers in Immunology*, 0. doi:10.3389/fimmu.2021.767319.
- Paardekooper, L.M., Bendix, M.B., Ottria, A., de Haer, L.W., ter Beest, M., Radstake, T.R.D.J., et al. (2018) 'Hypoxia potentiates monocyte-derived dendritic cells for release of tumor necrosis factor α via MAP3K8', *Bioscience Reports*, 38(6), p. BSR20182019. doi:10.1042/BSR20182019.
- Panayiotou, R., Miralles, F., Pawlowski, R., Diring, J., Flynn, H.R., Skehel, M., et al. (2016) 'Phosphorylation acts positively and negatively to regulate MRTF-A subcellular localisation and activity', *eLife*, 5, p. e15460. doi:10.7554/eLife.15460.
- Papaseit, C., Pochon, N. and Tabony, J. (2000) 'Microtubule self-organization is gravity-dependent', *Proceedings of the National Academy of Sciences*, 97(15), pp. 8364–8368. doi:10.1073/pnas.140029597.
- Patel, S. (2020) 'The effects of microgravity and space radiation on cardiovascular health: From low-Earth orbit and beyond', *IJC Heart & Vasculature*, 30, p. 100595. doi:10.1016/j.ijcha.2020.100595.
- Pathak, M.M., Tran, T., Hong, L., Joós, B., Morris, C.E. and Tombola, F. (2016) 'The Hv1 proton channel responds to mechanical stimuli', *The Journal of General Physiology*, 148(5), pp. 405–418. doi:10.1085/jgp.201611672.
- Paulsen, K., Tauber, S., Dumrese, C., Bradacs, G., Simmet, D.M., Gözl, N., et al. (2015) 'Regulation of ICAM-1 in Cells of the Monocyte/Macrophage System in Microgravity', *BioMed Research International*, 2015, p. e538786. doi:10.1155/2015/538786.
- Paulsen, K., Tauber, S., Goelz, N., Simmet, D.M., Engeli, S., Birlem, M., et al. (2014) 'Severe disruption of the cytoskeleton and immunologically relevant surface molecules in a human macrophageal cell line in microgravity—Results of an in vitro experiment on board of the Shenzhou-8 space mission', *Acta Astronautica*, 94(1), pp. 277–292. doi:10.1016/j.actaastro.2013.06.007.
- Poon, C. (2020) 'Factors implicating the validity and interpretation of mechanobiology studies in simulated microgravity environments', *Engineering Reports*, 2(10), p. e12242. doi:10.1002/eng2.12242.
- Qi, Y.-X., Yao, Q.-P., Huang, K., Shi, Q., Zhang, P., Wang, G.-L., et al. (2016) 'Nuclear envelope proteins modulate proliferation of vascular smooth muscle cells during cyclic stretch application', *Proceedings of the National Academy of Sciences*, 113(19), pp. 5293–5298. doi:10.1073/pnas.1604569113.
- Ramsey, I.S., Ruchti, E., Kaczmarek, J.S. and Clapham, D.E. (2009) 'Hv1 proton channels are required for high-level NADPH oxidase-dependent superoxide production during the phagocyte respiratory burst', *Proceedings of the National Academy of Sciences*, 106(18), pp. 7642–7647. doi:10.1073/pnas.0902761106.
- Ran, F., An, L., Fan, Y., Hang, H. and Wang, S. (2016) 'Simulated microgravity potentiates generation of reactive oxygen species in cells', *Biophysics Reports*, 2(5), pp. 100–105. doi:10.1007/s41048-016-0029-0.
- Record, J., Saeed, M.B., Venit, T., Percipalle, P. and Westerberg, L.S. (2021) 'Journey to the Center of the Cell: Cytoplasmic and Nuclear Actin in Immune Cell Functions', *Frontiers in Cell and Developmental Biology*, 9, p. 682294. doi:10.3389/fcell.2021.682294.
- Romero, L.O., Massey, A.E., Mata-Daboin, A.D., Sierra-Valdez, F.J., Chauhan, S.C., Cordero-Morales, J.F., et al. (2019) 'Dietary fatty acids fine-tune Piezo1 mechanical response', *Nature Communications*, 10(1), p. 1200. doi:10.1038/s41467-019-09055-7.
- Ronkina, N., Lafera, J., Kotlyarov, A. and Gaestel, M. (2016) 'Stress-dependent phosphorylation of myocardin-related transcription factor A (MRTF-A) by the p38(MAPK)/MK2 axis', *Scientific Reports*, 6, p. 31219. doi:10.1038/srep31219.

- Ronzier, E., Laurenson, A.J., Manickam, R., Liu, S., Saintilma, I.M., Schrock, D.C., et al. (2022) 'The Actin Cytoskeleton Responds to Inflammatory Cues and Alters Macrophage Activation', *Cells*, 11(11), p. 1806. doi:10.3390/cells11111806.
- Rubio, J.M., Astudillo, A.M., Casas, J., Balboa, M.A. and Balsinde, J. (2018) 'Regulation of Phagocytosis in Macrophages by Membrane Ethanolamine Plasmalogens', *Frontiers in Immunology*, 9, p. 1723. doi:10.3389/fimmu.2018.01723.
- Sakai, Y., Yamamori, T., Yoshikawa, Y., Bo, T., Suzuki, M., Yamamoto, K., et al. (2018) 'NADPH oxidase 4 mediates ROS production in radiation-induced senescent cells and promotes migration of inflammatory cells', *Free Radical Research*, 52(1), pp. 92–102. doi:10.1080/10715762.2017.1416112.
- Schaefer, A., Te Riet, J., Ritz, K., Hoogenboezem, M., Anthony, E.C., Mul, F.P.J., et al. (2014) 'Actin-binding proteins differentially regulate endothelial cell stiffness, ICAM-1 function and neutrophil transmigration', *Journal of Cell Science*, 127(Pt 20), pp. 4470–4482. doi:10.1242/jcs.154708.
- Schuch, K., Wanko, B., Ambroz, K., Castelo-Rosa, A., Moreno-Viedma, V., Grün, N.G., et al. (2016) 'Osteopontin affects macrophage polarization promoting endocytic but not inflammatory properties', *Obesity*, 24(7), pp. 1489–1498. doi:10.1002/oby.21510.
- Shanmugarajan, S., Zhang, Y., Moreno-Villanueva, M., Clanton, R., Rohde, L.H., Ramesh, G.T., et al. (2017) 'Combined Effects of Simulated Microgravity and Radiation Exposure on Osteoclast Cell Fusion', *International Journal of Molecular Sciences*, 18(11), p. 2443. doi:10.3390/ijms18112443.
- Sharma, S., Goswami, R., Merth, M., Cohen, J., Lei, K.Y., Zhang, D.X., et al. (2017) 'TRPV4 ion channel is a novel regulator of dermal myofibroblast differentiation', *American Journal of Physiology-Cell Physiology*, 312(5), pp. C562–C572. doi:10.1152/ajpcell.00187.2016.
- Shi, L., Tian, H., Wang, P., Li, L., Zhang, Z., Zhang, J., et al. (2021) 'Spaceflight and simulated microgravity suppresses macrophage development via altered RAS/ERK/NFκB and metabolic pathways', *Cellular & Molecular Immunology*, 18(6), pp. 1489–1502. doi:10.1038/s41423-019-0346-6.
- Shirato, K. and Sato, S. (2022) 'Macrophage Meets the Circadian Clock: Implication of the Circadian Clock in the Role of Macrophages in Acute Lower Respiratory Tract Infection', *Frontiers in Cellular and Infection Microbiology*, 12. Available at: <https://www.frontiersin.org/article/10.3389/fcimb.2022.826738> (Accessed: 29 May 2022).
- Sianati, S., Schroeter, L., Richardson, J., Tay, A., Lamandé, S.R. and Poole, K. (2021) 'Modulating the Mechanical Activation of TRPV4 at the Cell-Substrate Interface', *Frontiers in Bioengineering and Biotechnology*, 8, p. 1527. doi:10.3389/fbioe.2020.608951.
- Sidorenko, E., Sokolova, M., Pennanen, A.P., Kyheröinen, S., Posern, G., Foisner, R., et al. (2022) 'Lamina-associated polypeptide 2α is required for intranuclear MRTF-A activity', *Scientific Reports*, 12(1), p. 2306. doi:10.1038/s41598-022-06135-5.
- Simmet, D. (2013) 'Biotechnology for the Investigation of the Monocyte-Macrophage-System in Microgravity and Space', *Recent Patents on Space Technology*, pp. 48–63.
- Smith, J.K. (2020) 'Microgravity, Bone Homeostasis, and Insulin-Like Growth Factor-1', *Applied Sciences*, 10(13), p. 4433. doi:10.3390/app10134433.
- Smith, J.T., Willey, N.J. and Hancock, J.T. (2012) 'Low dose ionizing radiation produces too few reactive oxygen species to directly affect antioxidant concentrations in cells', *Biology Letters*, 8(4), pp. 594–597. doi:10.1098/rsbl.2012.0150.
- Sun, W., Chi, S., Li, Yuheng, Ling, S., Tan, Y., Xu, Y., et al. (2019) 'The mechanosensitive Piezo1 channel is required for bone formation', *eLife*. Edited by C.J. Rosen, H.C. Dietz, V. Sherk, and N.A. Haelterman, 8, p. e47454. doi:10.7554/eLife.47454.
- Sun, Y., Kuang, Y. and Zuo, Z. (2021) 'The Emerging Role of Macrophages in Immune System Dysfunction under Real and Simulated Microgravity Conditions', *International Journal of Molecular Sciences*, 22(5), p. 2333. doi:10.3390/ijms22052333.
- Tauber, S., Lauber, B.A., Paulsen, K., Layer, L.E., Lehmann, M., Hauschild, S., et al. (2017) 'Cytoskeletal stability and metabolic alterations in primary human macrophages in long-term microgravity', *PLOS ONE*, 12(4), p. e0175599. doi:10.1371/journal.pone.0175599.

- Thiel, C.S., Tauber, S., Lauber, B., Polzer, J., Seebacher, C., Uhl, R., et al. (2019) 'Rapid Morphological and Cytoskeletal Response to Microgravity in Human Primary Macrophages', *International Journal of Molecular Sciences*, 20(10), p. 2402. doi:10.3390/ijms20102402.
- Thiel, C.S., Vahlensieck, C., Bradley, T., Tauber, S., Lehmann, M. and Ullrich, O. (2021) 'Metabolic Dynamics in Short- and Long-Term Microgravity in Human Primary Macrophages', *International Journal of Molecular Sciences*, 22(13), p. 6752. doi:10.3390/ijms22136752.
- Thiel, C.S., de Zélicourt, D., Tauber, S., Adrian, A., Franz, M., Simmet, D.M., et al. (2017) 'Rapid adaptation to microgravity in mammalian macrophage cells', *Scientific Reports*, 7(1), p. 43. doi:10.1038/s41598-017-00119-6.
- Thompson, M., Woods, K., Newberg, J., Oxford, J.T. and Uzer, G. (2020) 'Low-intensity vibration restores nuclear YAP levels and acute YAP nuclear shuttling in mesenchymal stem cells subjected to simulated microgravity', *npj Microgravity*, 6(1), pp. 1–11. doi:10.1038/s41526-020-00125-5.
- Tilghman, R.W. and Hoover, R.L. (2002) 'E-selectin and ICAM-1 are incorporated into detergent-insoluble membrane domains following clustering in endothelial cells', *FEBS letters*, 525(1–3), pp. 83–87. doi:10.1016/s0014-5793(02)03070-3.
- Vahlensieck, C., Thiel, C.S., Pöschl, D., Bradley, T., Krammer, S., Lauber, B., et al. (2022) 'Post-Transcriptional Dynamics is Involved in Rapid Adaptation to Hypergravity in Jurkat T Cells', *Frontiers in Cell and Developmental Biology*, 10. Available at: <https://www.frontiersin.org/articles/10.3389/fcell.2022.933984> (Accessed: 16 July 2022).
- Venit, T., El Said, N.H., Mahmood, S.R. and Percipalle, P. (2021) 'A dynamic actin-dependent nucleoskeleton and cell identity', *Journal of Biochemistry*, 169(3), pp. 243–257. doi:10.1093/jb/mvaa133.
- Vogel, J., Thiel, C.S., Tauber, S., Stockmann, C., Gassmann, M. and Ullrich, O. (2019) 'Expression of Hypoxia-Inducible Factor 1 α (HIF-1 α) and Genes of Related Pathways in Altered Gravity', *International Journal of Molecular Sciences*, 20(2), p. 436. doi:10.3390/ijms20020436.
- Wang, C., Chen, H., Luo, H., Zhu, L., Zhao, Yang, Tian, H., et al. (2015) 'Microgravity activates p38 MAPK-C/EBP β pathway to regulate the expression of arginase and inflammatory cytokines in macrophages', *Inflammation Research*, 64(5), pp. 303–311. doi:10.1007/s00011-015-0811-3.
- Wang, C., Luo, H., Zhu, L., Yang, F., Chu, Z., Tian, H., et al. (2014) 'Microgravity inhibition of lipopolysaccharide-induced tumor necrosis factor- α expression in macrophage cells', *Inflammation Research*, 63(1), pp. 91–98. doi:10.1007/s00011-013-0676-2.
- Wang, J., Han, C., Lu, Z., Ge, P., Cui, Y., Zhao, D., et al. (2020) 'Simulated microgravity suppresses MAPK pathway-mediated innate immune response to bacterial infection and induces gut microbiota dysbiosis', *The FASEB Journal*, 34(11), pp. 14631–14644. doi:10.1096/fj.202001428R.
- Wang, Y., Wang, G.Z., Rabinovitch, P.S. and Tabas, I. (2014) 'Macrophage Mitochondrial Oxidative Stress Promotes Atherosclerosis and Nuclear Factor- κ B-Mediated Inflammation in Macrophages', *Circulation Research*, 114(3), pp. 421–433. doi:10.1161/CIRCRESAHA.114.302153.
- Wang, Z., Brandt, S., Medeiros, A., Wang, S., Wu, H., Dent, A., et al. (2015) 'MicroRNA 21 Is a Homeostatic Regulator of Macrophage Polarization and Prevents Prostaglandin E2-Mediated M2 Generation', *PLoS ONE*, 10(2), p. e0115855. doi:10.1371/journal.pone.0115855.
- Wu, Q., Allouch, A., Martins, I., Modjtahedi, N., Deutsch, E. and Perfettini, J.-L. (2017) 'Macrophage biology plays a central role during ionizing radiation-elicited tumor response', *Biomedical Journal*, 40(4), pp. 200–211. doi:10.1016/j.bj.2017.06.003.
- Wubshet, N.H., Arreguin-Martinez, E., Nail, M., Annamalai, H., Koerner, R., Rouseva, M., et al. (2021) 'Simulating microgravity using a random positioning machine for inducing cellular responses to mechanotransduction in human osteoblasts', *Review of Scientific Instruments*, 92(11), p. 114101. doi:10.1063/5.0056366.
- Xiong, X., Li, W., Nam, J. and Ma, K. (2021) 'Integrin signaling via actin cytoskeleton activates MRTF/SRF to entrain circadian clock'. bioRxiv, p. 2021.08.12.456061. doi:10.1101/2021.08.12.456061.
- Yang, P.-Y., Almofti, M.R., Lu, L., Kang, H., Zhang, J., Li, T.-J., et al. (2005) 'Reduction of Atherosclerosis in Cholesterol-Fed Rabbits and Decrease of Expressions of Intracellular Adhesion Molecule-1 and Vascular

Endothelial Growth Factor in Foam Cells by a Water-Soluble Fraction of Polygonum multiflorum', *Journal of Pharmacological Sciences*, 99(3), pp. 294–300. doi:10.1254/jphs.FP0050333.

Yang, X., Sun, L.-W., Du, C.-F., Wu, X.-T. and Fan, Y.-B. (2018) 'Finite Element Analysis of Osteocytes Mechanosensitivity Under Simulated Microgravity', *Microgravity Science and Technology*, 30(4), pp. 469–481. doi:10.1007/s12217-018-9613-x.

Yang, Y., Yang, G., Yu, L., Lin, L., Liu, L., Fang, M., et al. (2020) 'An Interplay Between MRTF-A and the Histone Acetyltransferase TIP60 Mediates Hypoxia-Reoxygenation Induced iNOS Transcription in Macrophages', *Frontiers in Cell and Developmental Biology*, 8, p. 484. doi:10.3389/fcell.2020.00484.

Zhang, L., Li, H.-L., Zhang, D.-D. and Cui, X.-C. (2021) 'Therapeutic effects of myocardin-related transcription factor A (MRTF-A) knockout on experimental mice with nonalcoholic steatohepatitis induced by high-fat diet', *Human & Experimental Toxicology*, 40(10), pp. 1634–1645. doi:10.1177/09603271211002886.

Zhong, H., Lin, H., Pang, Q., Zhuang, J., Liu, X., Li, X., et al. (2021) 'Macrophage ICAM-1 functions as a regulator of phagocytosis in LPS induced endotoxemia', *Inflammation Research*, 70(2), pp. 193–203. doi:10.1007/s00011-021-01437-2.

Zhou, H., Xue, Y., Dong, L. and Wang, C. (2021) 'Biomaterial-based physical regulation of macrophage behaviour', *Journal of Materials Chemistry B*, 9(17), pp. 3608–3621. doi:10.1039/D1TB00107H.