Shear Stress Induces a Pro-Inflammatory Phenotype in Human Monocyte-Derived Macrophages

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Abbreviated title

Shear stress-induced MDM phenotype

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Abstract

Macrophages are innate immune cells that are known for their diverse phenotypes and plasticity. They switch between pro-inflammatory and anti-inflammatory/pro-fibrotic phenotypes depending on surrounding microenvironmental cues. Although much research has been focused on their response to biochemical cues and mechanical signals, there is a paucity of knowledge surrounding macrophage behavior in response to shear stress even though shear stress has been implicated to affect macrophage phenotype in diseases such as atherosclerosis. It is also difficult to determine the specific signals that macrophages release in response to shear *in vivo*, necessitating a more controlled environment using an *in vitro* model. We achieved this by applying varying magnitudes of shear stress on monocyte-derived macrophages (MDMs) using a cone-and-plate viscometer and evaluated changes in genetic expression, protein expression, and cytokine secretion. Interestingly, we found that prolonged shear exposure induced a pro-inflammatory M1 phenotype. These findings have implications in applications such as *in situ* vascular graft design, where macrophages are exposed to shear and have been shown to affect graft resorption. Future studies should evaluate longer timepoints and higher shear rates.

Introduction

Macrophages are innate immune cells that readily adopt a wide spectrum of phenotypes regulated by the biochemical cues from their surrounding microenvironment. Upon exposure to pro-inflammatory signals such as interferon-γ (IFN-γ) and lipopolysaccharide (LPS), macrophages classically activate into the pro-inflammatory M1 phenotype. By contrast, exposure to anti-inflammatory signals such as interleukin (IL)-4 and IL-13 incurs alternative activation into the anti-inflammatory M2 phenotype. Macrophage plasticity enables them to play a critical role in wound healing regulation. M1 macrophages dominate the pro-inflammatory phase of wound healing, clearing cellular debris and secreting pro-inflammatory cytokines and chemokines, such as tumor necrosis factor-α (TNF-α), IL-1β, IL-6, IL-12, and IL-23 (1). Alternatively, M2 macrophages are heavily involved in wound resolution and fibrosis and secrete factors such as IL-10 and transforming growth factor-β (TGF-β) that dampen the inflammatory response (2). Furthermore, M1 and M2 macrophages display distinct morphological differences. M1 macrophages exhibit the traditional "fried egg" morphology, whereas M2 macrophages are elongated and spindle-like (3, 4). Biochemical signals cause macrophages to respond not only genetically, but also morphologically.

Although much of the current research has focused on the role of cytokines and chemokines in regulating macrophage function, the role of the mechanical microenvironment has received considerably less attention. Macrophages respond to their surrounding mechanical microenvironment through a process called mechanotransduction. They sense mechanical stimuli, such as stiffness and topography, and convert them into biochemical signals. Recent works have shown that changes in stiffness regulate the differentiation and function of macrophages (5-7). Specifically, increased stiffness, as seen in fibrotic tissue, regulates macrophage polarization towards an anti-inflammatory M2 phenotype (7). Interestingly, macrophages in fibrotic tissue exhibit an elongated morphology correlating with the M2 phenotype (3). Findings have also shown that macrophages can be induced to polarize by directly modulating their morphology. For example, McWhorter et al. engineered cell culture substrates to control macrophage morphology and concluded that elongating the macrophage morphology upregulates M2 markers and reduces secretion of pro-inflammatory cytokines (3). Aside from stiffness and topology, macrophages can be influenced by dynamic mechanical loading, such as stretch and compression. Studies have shown that in human monocyte-derived macrophages (MDMs), cyclic stretch induces the expression of matrix metalloproteinase (MMP)-1 and MMP-3 and encourages their differentiation into osteoclasts (8, 9). Overall, these investigations demonstrate that macrophages are not only influenced by cellular signals, but also by the mechanical forces imposed upon them.

Although researchers have begun to investigate the effect of various mechanical stimuli on macrophages, there is a paucity of articles over their response to shear stress. Recent work investigating macrophage behavior in shear stress conditions have primarily been focused on atherosclerotic plaque formation (1, 10, 11). Specifically, *in vivo* mouse models of atherosclerosis have shown that high shear is atheroprotective, dominated by M2 macrophages, and correlated with plaque stability, whereas low shear is atheroinducive, prevailed by M1 macrophages, and vulnerable to plaque rupture (10, 12, 13). However, it is difficult to tease out the specific effects of fluid shear stress on macrophages *in vivo* considering that other factors such as stiffness and

cellular signaling influence macrophage function. Other studies have evaluated hemodynamic loading on macrophages in the context of biomaterial-driven *in* situ vascular regeneration and shown that macrophage activation is augmented upon exposure to shear stress (14-16). These studies demonstrate that macrophages are responding to changes in hemodynamics, however there is limited knowledge in the biochemical cues that macrophages release in response to these changes. Therefore, in this study, we propose an *in vitro* model system that characterizes macrophage behavior under various rates of physiological shear (15 dynes/cm^2 and 35 dynes/cm^2) (17). Human monocyte-derived macrophages (MDMs) were exposed to 15 dynes/cm^2 and 35 dynes/cm^2 shear stress over a period of 3, 24, and 48 hours using a cone-and-plate viscometer. MDMs were then evaluated for genetic expression, protein expression, and cytokine secretion.

Materials and Methods

Primary human monocyte-derived macrophages

Human monocytes were isolated from donor buffy coats received from the Gulf Coast Regional Blood Center (Houston, TX). The buffy coats were layered on top of 15 mL of Histopaque (1.077 g/mL; Sigma-Aldrich, St. Louis, MO) and centrifuged at 400 x g for 30 minutes without brake. Following centrifugation, the peripheral blood mononuclear cells (PBMCs) were collected from the interphase, pooled with 3-4 donors, and washed twice with 1x PBS containing 5 mM EDTA. PBMCs were seeded onto tissue-culture treated flasks with the addition of macrophage colony stimulating factor (MCSF, 20 ng/µL; Novus Biologicals, Centennial, CO). After 24 hours, the floating lymphocytes were aspirated, and the flask was washed 2x with PBS to remove remaining lymphocytes, leaving attached monocytes. Monocytes were cultured for a subsequent 5-7 days in RPMI 1640 (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and MCSF (20 ng/µL). Media was replenished on days 3 and 5. On days 5-7, macrophages were harvested after incubation with Accutase (Sigma-Aldrich) for 15 minutes along with gentle cell scraping.

Shear stress application

Uniform laminar shear stress was applied to primary human MDMs using a cone-and-plate viscometer system. The cone was placed on top of a 60 mm petri dish seeded with a monolayer of MDMs which was then positioned on top of a controllable magnetic stir plate. Each petri dish was washed with PBS and supplemented with 3 mL of fresh MDM media (RPMI 1640 supplemented with 10% FBS and 1% penicillin/streptomycin) prior to shear exposure. The cone was then rotated at various speeds: 0 RPM, 280 RPM, and 660 RPM, which equated to a shear rate of 0 dynes/cm^2 (static), 15 dynes/cm^2 (physiological), and 35 dynes/cm^2 (pathological), respectively, using the governing wall shear stress equation:

$$\tau = \mu\left(\frac{\omega \cdot r}{r \cdot \alpha + \chi}\right)$$

where τ is the desired shear stress, µ is the dynamic viscosity (0.95 centistokes) of the media, ω is the angular velocity of the cone, r is the radius (30 mm) of the cone, α is the angle (0.5°) of the cone, and χ is the gap height (300 µm) between the cone and the cells. MDMs were exposed to

these shear rates at time points of 3, 24, and 48 hours, and phenotypes were then evaluated through flow cytometry and rt-qPCR. The 48-hour 35 dynes/cm^2 group was supplemented with an extra 1 mL of RPMI 1640 without serum to prevent the samples from drying out during the duration of the experiment.

Flow cytometry

After isolation and differentiation, MDMs were evaluated for purity through flow cytometry for expression of pan-macrophage marker, CD68. MDMs were harvested after 15 minutes of Accutase exposure through gentle scraping. For intracellular staining, MDMs were fixed with 4% paraformaldehyde for 20 minutes and permeabilized in PBS with 0.5% Tween20 and 1% FBS. Cells were then incubated with APC-conjugated mouse anti-human CD68 (BD Biosciences, Franklin Lakes, NJ) for 1 hour.

Following shear stress application, MDM protein expressions were evaluated through flow cytometry. Cells were stained with the following antibodies: FITC-conjugated mouse anti-human CD80 (BD Biosciences), PE-conjugated mouse anti-human CD163 (BD Biosciences), and APC-conjugated mouse anti-human CD206 (BD Biosciences). Blocking of non-specific binding was achieved with FBS. Flow cytometry data was acquired using the Sony MA900 multi-application cell sorter (Sony Biotechnology Inc, San Jose, CA) and was analyzed using FlowJo (FlowJo, LLC, Ashland, OR). Percent positive cells for each marker were gated to 1% of the negative control.

Viability staining and image quantification

Cell viability was quantified following shear stress application by incubating each sample in a solution containing 5 µL calcein AM and 20 µL ethidium homodimer-1 in 10 mL of PBS (Live/Dead Viability/Cytotoxicity Kit; Thermo Fisher, Waltham, MA) for 30 minutes at room temperature. Samples were imaged immediately via fluorescent microscopy.

RNA isolation, cDNA synthesis, and quantitative reverse transcription PCR

Following shear stress application, MDM phenotypes were evaluated through quantitative reverse transcription PCR (rt-qPCR). MDMs were harvested after 15 minutes of Accutase exposure through gentle scraping. RNA was extracted using the Direct-zol™ RNA Microprep kit (Zymo Research) according to the manufacturer's instructions. Briefly, MDMs were lysed with TRIzol (Thermo Fisher), mixed with an equal volume of 95% ethanol, and transferred to a Zymo-Spin™ IC Column in a collection tube. Samples were then incubated with DNase I for 15 minutes at room temperature, washed with RNA wash buffer, and eluted with 15 µL of DNase/RNase-free water. RNA purity ($A_{260/280} > 1.8$) was then assessed through the NanoDrop 2000 spectrophotometer (Thermo Fisher) and stored at -80°C until gene expression analysis. Complementary DNA (cDNA) was synthesized from purified RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA) and stored at -20°C until gene expression analysis. rt-qPCR was performed using the iTaq™ Universal Probes Supermix (Bio-Rad Laboratories, Hercules, CA) according to manufacturer's instructions with primers specific to human CCR7 (probe ID: Hs01013469_m1), MRC1 (Hs02832368_g1), TGFB1

(Hs00998133_m1), TNF (Hs00174128_m1), and GAPDH (Hs02786624_g1). Fold changes were first normalized to GAPDH and then to the control group.

Conditioned media analysis

After shear exposure, conditioned media secreted from MDMs were collected and centrifuged at 1000 x g for 5 minutes to remove cellular debris. The supernatant was then collected and stored at -80°C until cytokine analysis. Cytokines were analyzed using the MILLIPLEX® Human 48-Plex Cytokine Panel A kit (MilliporeSigma, Burlington, MA) on the Luminex system by a proteomics core (Baylor College of Medicine, Houston, TX) as per manufacturer's instructions. Briefly, samples were thawed on ice and centrifuged at 10000 x g for 5 mins. Samples and beads were incubated at 4°C overnight on a shaker for 16 hours. RPMI-1640 media was used as the blank. Two quality control samples were prepared, and a Logistic-5PL regression type was used to construct the standard curve of all the analytes.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 6 (GraphPad Software Inc., La Jolla, CA). A two-way analysis of variance (ANOVA) with post-hoc Tukey's testing was used to determine statistical significance ($p < 0.05$) for each set of conditions in this study. All experiments were independently repeated in triplicates.

Results

Shear stress exposure modulates MDM morphology

A cone-and-plate viscometer was used to apply varying shear rates (0 dynes/cm^2 (static), 15 dynes/cm^2, and 35 dynes/cm^2) to MDMs for a period of 3, 24, and 48 hours as shown in **Figure 1**. Post shear exposure, samples were live/dead stained to evaluate MDM morphology and cellular viability as shown in **Figure 2**. As shown in the fluorescent images, cell viability does not change over time or shear rate. In terms of morphology, no major differences were observed between the 15 and 35 dynes/cm^2 conditions over all time points. However, at 48 hours, MDMs exhibited a more spindles compared to 24 and 48 hours.

Shear stress upregulates the genetic expression of TNF-α in a time-dependent manner

After shear exposure, RNA was isolated from samples, and rt-qPCR was performed to evaluate the effect of shear on the genetic expression of MDMs. We investigated TNF, which codes for TNF-α, and CCR7 as markers for inflammation and M1 phenotype; and TGF-β1 and MRC1, which codes for CD206, as markers for fibrosis and M2 phenotype. The expression of TNF was most impacted at the 24-hour timepoint where increasing levels of shear promoted significant upregulation. Furthermore, within each shear condition, prolonged shear exposure significantly upregulated its expression compared to the static condition. However, expression was downregulated at 48 hours compared to 24 hours. CCR7 demonstrated a downward trend starting at 24 hours with significant downregulation at 48 hours with increasing shear. CCR7 also displayed

non-significant downward trends within each shear condition in response to prolonged time compared to the static control. TGF-β1 was significantly downregulated at 24 hours in the 35 dynes/cm^2 timepoint but otherwise remained relatively unaffected. MRC1 displayed an upward trend at 3 hours in response to increasing shear but shifted to decreasing levels by 24 and 48 hours. Within each shear condition however, prolonged exposure resulted in decreased MRC1 expression.

Shear stress promotes time-dependent pro-inflammatory cytokine secretion

Since genetic expression of TNF-α was considerably upregulated compared to other markers, we decided to analyze conditioned media for cytokine levels. After shear exposure, conditioned media was collected at each time point and shear rate and analyzed for cytokine secretion using a 48-plex cytokine panel. The results shown in **Figure 4 and 5** demonstrate that both shear rate and time affects cellular signaling. Interestingly, within each shear condition, increasing time overwhelmingly correlated with increased pro-inflammatory cytokine production, i.e 24 hours showed more secretion compared to 3 hours, and 48 hours showed more compared to 24 hours. IFN-γ, granulocyte-colony stimulating factor (G-CSF), and numerous chemokines (IL-8, IL-15, monocyte chemoattractant protein-1 (MCP-1), macrophage-derived chemokine (MDC), and chemokine ligand 9 (CXCL9)), significantly increased in response to prolonged time. In contrast, chemokines MIP-1α and MIP-1β were significantly downregulated as time increased in the static condition. Secretion of profibrotic cytokines, however, had varied results. Epidermal growth factor (EGF) was significantly upregulated at 24 hours compared to 3 hours in the static condition but was downregulated after 48 hours. Furthermore, it remained significantly downregulated in the 15 and 35 dynes/cm^2 group as time increased. Fibrotic growth factor-2 (FGF-2) mostly remained unchanged over time but was significantly decreased at 24 hours compared to 3 hours. IL-22 was significantly downregulated at 48 hours compared to 24 hours in the static condition. This trend continued within the 15 dynes/cm^2 condition but shifted towards an upward trend in the 35 dynes/cm^2 condition with increasing time. Vascular endothelial growth factor-A (VEGF-A) and platelet derived growth factor-AA (PDGF-AA) were upregulated in the 15 and 35 dynes/cm^2 groups but remained unchanged in the static condition.

Increasing shear, however, stimulated contrasting effects within different timepoints. At 3 hours, CXCL9 and TNF-α were significantly downregulated in response to increasing shear. However, at 24 and 48 hours, these cytokines were upregulated following increased shear. IFN-γ, IL-8, and MDC also experienced the same trend, although non-significant. Moreover, increased shear did not affect MCP-3 at 3 hours but upregulated its expression at 24 and 48 hours in the 35 dyne/cm^2 condition. MCP-1, however, exhibited decreased expression in the 15 dyne/cm^2 condition and became significantly downregulated in the 35 dyne/cm^2 condition at 3 hours. By 24 hours, however, MCP-1 displays a non-significant upregulation with increased shear but plateaus by 48 hours. RANTES was not affected by increased shear at 3 hours but was significantly upregulated with increasing shear at 24 hours and plateaus at 48 hours. MIP-1α and MIP-1β was significantly downregulated at 3 hours with increasing shear and was completely suppressed regardless of shear rate at 24 and 48 hours.

Eotaxin and fractalkine, potent chemoattractants for eosinophils and monocytes/T cells, respectively, were upregulated with increased shear at every timepoint, however, it was most significant at the 3 hour and 48-hour timepoints. Notably, fractalkine was not expression in the static conditions at any timepoint. Moreover, there were no proinflammatory cytokines that were significantly suppressed with increasing shear across every single timepoint although G-CSF and CXCL1 exhibited suppressive trends.

Looking at the effects of increased shear on profibrotic cytokine release within each timepoint, EGF was significantly upregulated in response to increased shear at 3 hours but was increasingly suppressed at 24 and 48 hours. This result is logical given the previously discussed downregulation of pro-inflammatory cytokines at 3 hours and upregulation at later timepoints. IL-10, a major cytokine secreted by M2 macrophages, was significantly downregulated following increased shear at 3 hours, and continued the downward trend at 24 and 48 hours. PDGF-AA and VEGF-A remained unchanged within the 3-hour timepoint but was significantly upregulated with increasing shear at 24 and 48 hours. IL-22 expression was significantly decreased with increased shear at 24 hours.

Prolonged shear exposure suppresses MDM surface marker expression

Protein expression was also evaluated after shear exposure to assess the effects of shear on surface marker expression as shown in **Figure 6 and 7**. We investigated CD80 as a marker for M1 phenotype, CD206 as a marker for M2a phenotype, and CD163 as a marker for M2c phenotype. Notably, no significant changes were observed for CD80, CD206, and CD163 within each timepoint in response to altered shear. However, prolonged exposure within each condition, including static, significantly decreased CD80 and CD163 expression. CD80 was significantly decreased in response to increased time in all groups whereas CD163 was significantly decreased in the 15 and 35 dynes/cm^2 conditions. Although CD206 generally displayed a downward trend in response to increased shear levels, expression increased with time at 24 hours.

Discussion

In order to evaluate macrophage behavior in response to shear stress, we subjected human MDMs to physiological levels of shear using a cone-and-plate viscometer over the course of 3, 24, and 48 hours and characterized their morphology, genetic expression, cytokine secretion, and protein expression. When investigating cellular morphology following shear exposure, MDMs were shown to exhibit more spindles at 48 hours in both shear groups compared to the static control. These results demonstrated that MDM morphology changed in response to a combination of shear magnitude and time. Furthermore, the live/dead stains indicated that cellular viability was not affected with shear exposure. Genetic changes following shear exposure were also evaluated. The predominant findings were that CCR7 and MRC1, respective M1 and M2 markers, were significantly downregulated in response to prolonged shear. However, TNF, a prominent M1 marker, was significantly upregulated at 24 hours by a large magnitude while TGF-β1, an M2 marker, was downregulated at 24 hours as shear increased. These results indicated that the MDMs may be primed towards an M1 phenotype following shear exposure and prompted us to evaluate cytokine release.

The results of the cytokine analysis indicate that MDMs produce complex signaling pathways in response to shear stress. When evaluating cytokine levels over time within each shear condition, an overwhelming number of pro-inflammatory cytokines were significantly upregulated as time increased. IFN-γ, a potent activator of M1 macrophages, G-CSF, a neutrophil regulator, and numerous chemokines (IL-8, IL-15, MCP-1, MDC, and CXCL9), significantly increased in response to prolonged shear exposure. Chemokines MIP-1α and MIP-1β are significantly downregulated in response to increased time in the static condition and exhibit the same trend following exposure to 15 dynes/cm^2 of shear. The conflicting results between chemokine expressions over time can be contributed to the possibility of different mechanisms regulating these chemokines. For example, the mechanisms promoting MIP-1α expression in macrophages is not well understood although the TLR4-IRF3 pathway is reported to be involved (18). Furthermore, MIP-1α release by macrophages is reported to be downregulated by IFN-γ *in vitro* (19).

When analyzing the secretion of profibrotic cytokines over time within each group, EGF was significantly downregulated in response to prolonged shear in the 35 dynes/cm^2 condition at 24 and 48 hours. These results are consistent with the upregulation of pro-inflammatory cytokines with prolonged shear. Additionally, VEGF-A and PDGF-AA were upregulated over longer time points in both sheared conditions. However, although both growth factors are heavily regulated by M2 macrophages and involved in cell differentiation, proliferation, and angiogenesis, they have both, notably, been reported to stimulate monocyte/macrophage chemotaxis (20). Furthermore, studies have also reported that M1 macrophages secrete the highest levels of VEGF-A compared to other macrophage phenotypes (21). These results speculate that VEGF-A may be playing a pro-inflammatory role in response to shear stress.

When evaluating cytokine levels in response to increasing shear within each timepoint, results were much more varied. The initial downregulation of proinflammatory cytokines CXCL9, TNF-α, IFN-γ, IL-8, MCP-1 and MDC at early timepoints in response increasing shear and their subsequent upregulation at later timepoints is compelling. We speculate that MDMs are adjusting to the initial onset of shear exposure and are priming for prolonged shear exposure. The delayed upregulation of MCP-3 and RANTES is also speculated to be attributed to this priming stage. The upregulation of these cytokines in response to increasing magnitudes of shear at longer timepoints is indicative of a chemotactic response. TNF-α and IFN-γ are potent activators of MDMs, specifically inducing M1 polarization (22). Furthermore, CXCL9, IL-8, MCP-1, MDC, MCP-3, and RANTES are chemokines that potently attract a wide variety of leukocytes including neutrophils, T-cells, monocytes, and macrophages (23-28). Moreover, CXCL9 is known to promote differentiation and multiplication of leukocytes and cause tissue extravasation (23). It's also attributed to diseases such as atherosclerosis where changes in hemodynamics directly affects macrophage phenotype and disease outcome (29). To further support MDM chemotactic response following shear exposure, eotaxin and fractalkine were shown to be upregulated with increased shear at every timepoint. These are two potent chemoattractants for eosinophils and monocytes/T cells, respectively (30, 31). Interestingly, fractalkine was not expressed in the static condition at any timepoint, indicating that shear stress exposure definitively induces macrophage-derived fractalkine secretion. When evaluating profibrotic markers, EGF was downregulated while VEGF-A and PDGF-AA were upregulated at later timepoints in response to increasing magnitudes of

shear. These results demonstrate that shear magnitude plays a major role in driving MDM phenotype.

We next investigated the effect of shear stress on MDM surface marker expression. Overall, shear stress seemed to suppress the expression of surface markers. CD80 and CD163 were significantly downregulated with prolonged shear exposure. CD206 was also downregulated aside from the 24-hour timepoint where upregulation was observed. We speculate that increased exposure time to elevated levels of shear could be damaging the surface receptors and causing this downregulation. It would be interesting to probe for intracellular markers such as CD68, a pan macrophage marker.

This is the first study to evaluate the effects of shear stress on MDM phenotype *in vitro*. MDMs play a crucial role in the development of atherosclerosis, where it has been reported that MDM phenotype changes depending on their location in the plaque. Different regions of the plaque are also exposed to varying rates of hemodynamic shear stress. These studies indicate that MDM phenotype is directly affected by the magnitude of shear stress, however it is difficult to determine the specific biomechanical cues that macrophages express *in vivo*. Our results indicate that prolonged shear stress exposure promotes a pro-inflammatory response by MDMs.

Conclusions

The specific effect of shear stress on macrophages is not well understood. We sought to investigate this effect by characterizing MDM genetic expression, protein expression, and cytokine secretion after shear exposure. We have demonstrated that prolonged shear exposure induces a pro-inflammatory response from MDMs, indicative of an M1 phenotype. The results of this work should be considered when designing *in situ* vascular grafts where MDMs are exposed to hemodynamic shear stress which can affect the rate of graft resorption and tissue promotion. Future work should investigate longer timepoints since fibrosis is the product of most chronic inflammatory diseases (32).

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Author Contributions

EJ drafted the manuscript. EJ, GK, and KP performed the experiments. All authors critically edited the manuscript.

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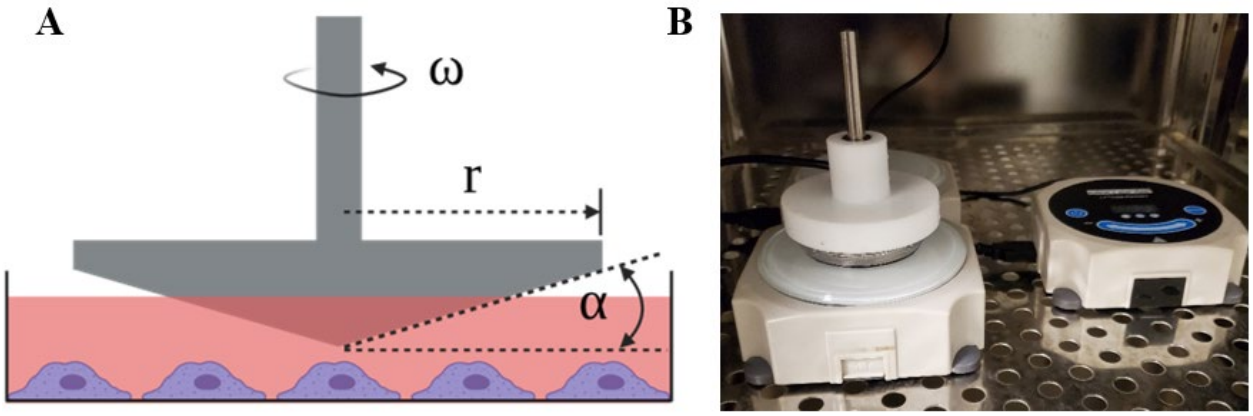

Figure 1. Cone-and-plate system. (A) Schematic of the cone-and-plate system where MDMs are attached to the bottom of the petri dish and a cone is loaded on top. (B) Setup of the cone-and-plate system in the incubator. The right side shows the controller that sets the RPM of the system.

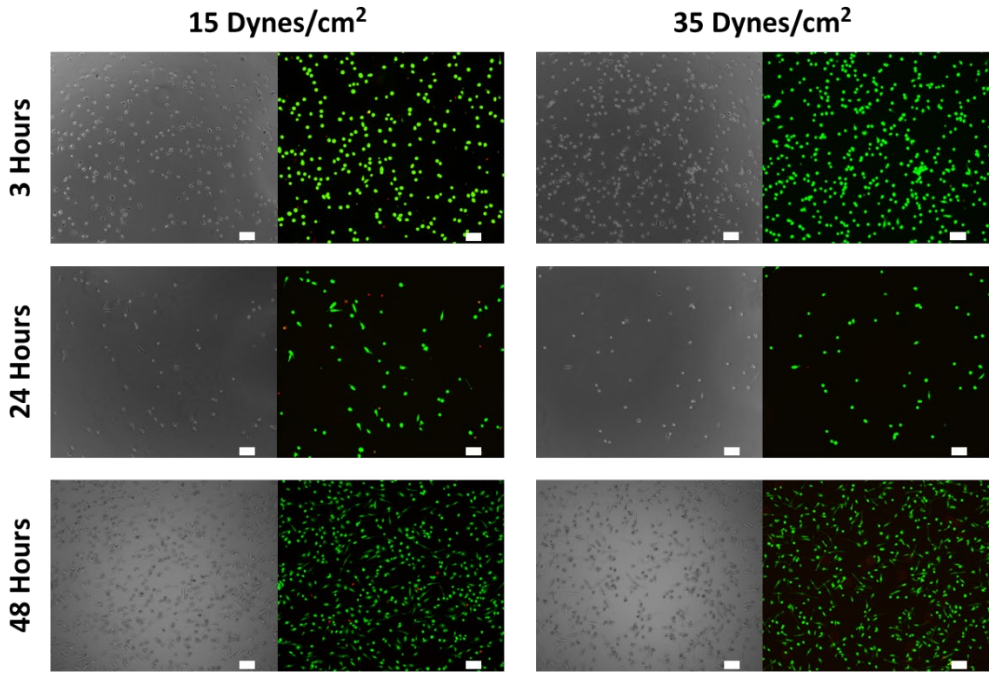

Figure 2. Brightfield images and live/dead staining following shear exposure. Each image was taken immediately after shear exposure at 3, 24, and 48 hours and is representative of all data collected. Scale bar = 100 µm.

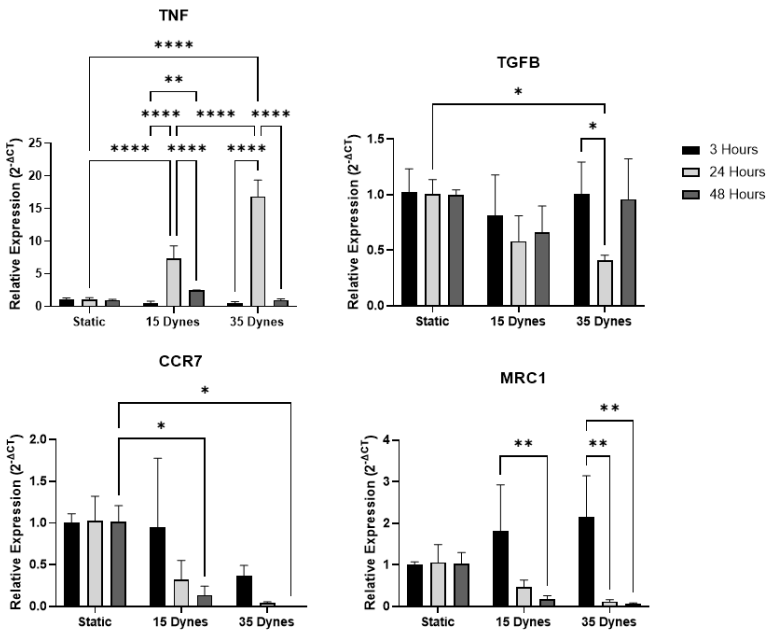

Figure 3. Genetic expression of TNF, TGF-β, CCR7, and MRC1 from MDMs cultured under static, 15 dynes/cm^2, and 35 dynes/cm^2 conditions for a period of 3, 24, and 48 hours. Data is expressed as mean ± SD, n ≥ 3.

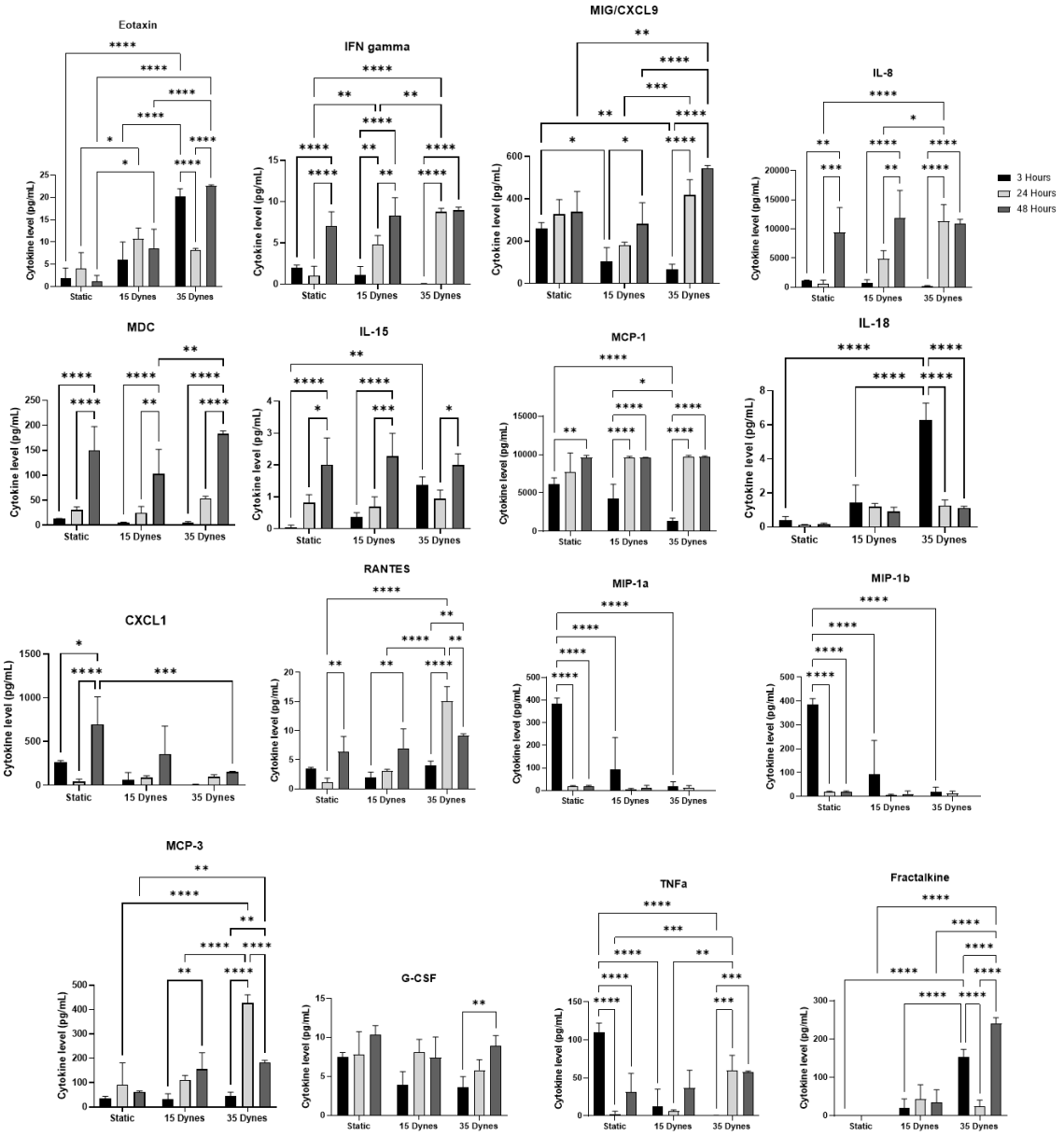

Figure 4. Pro-inflammatory cytokine secretion from MDMs cultured under static, 15 dynes/cm^2, and 35 dynes/cm^2 conditions for a period of 3, 24, and 48 hours. Data is expressed as mean ± SD, n = 4.

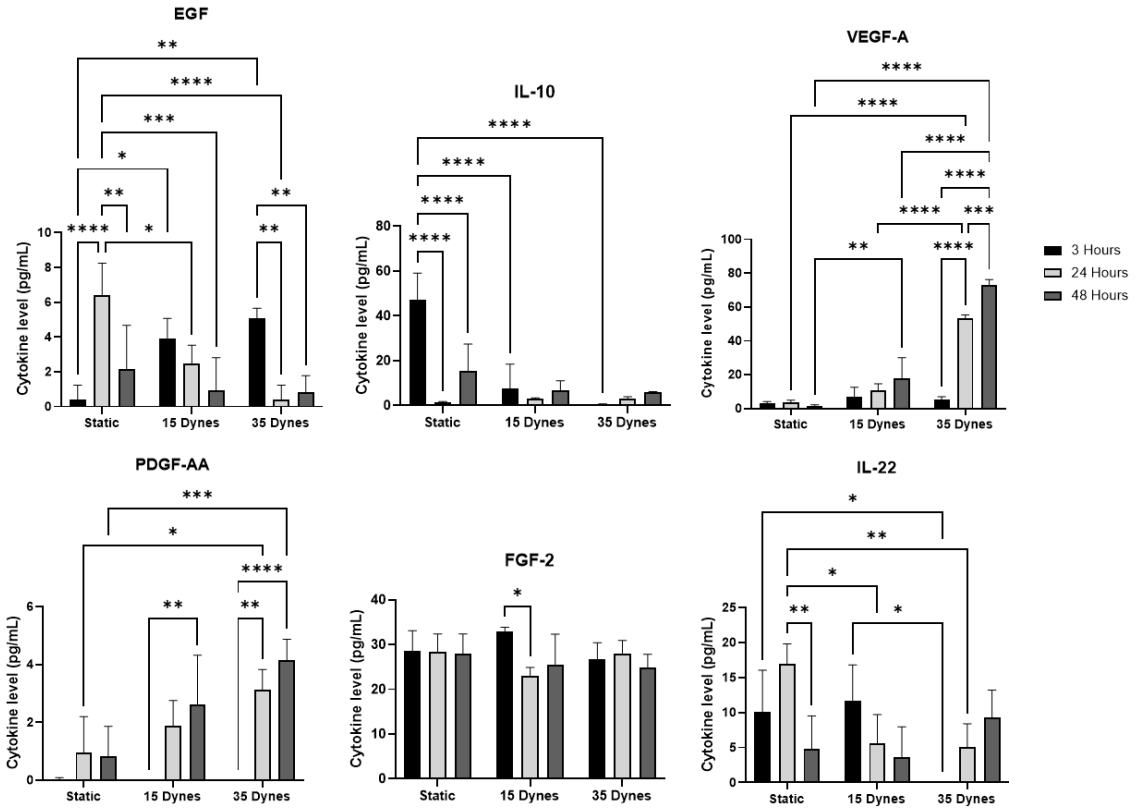

Figure 5. Anti-inflammatory cytokine secretion from MDMs cultured under static, 15 dynes/cm^2, and 35 dynes/cm^2 conditions for a period of 3, 24, and 48 hours. Data is expressed as mean ± SD, n = 4.

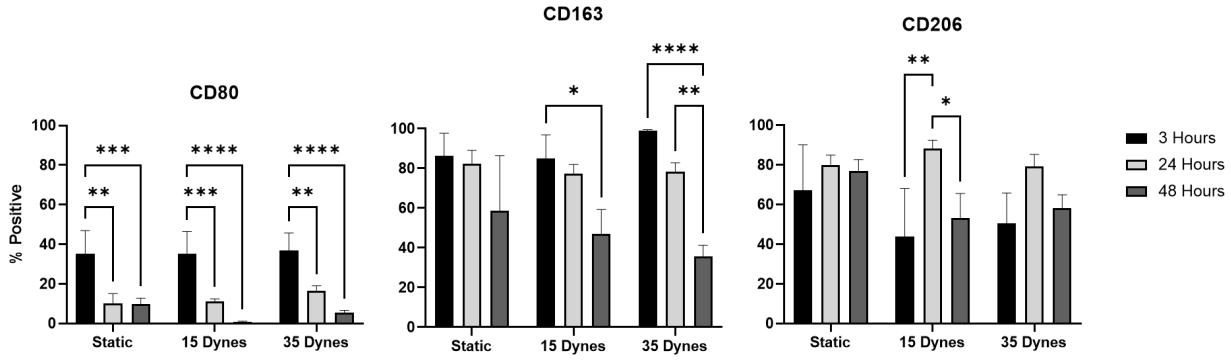

Figure 6. Percent positive protein expression through flow cytometry. CD80, CD163, and CD206 expression was evaluated through flow cytometry following shear exposure. Positive expression is gated to 1% of negative control. Data is represented as mean ± SD, n ≥ 3.

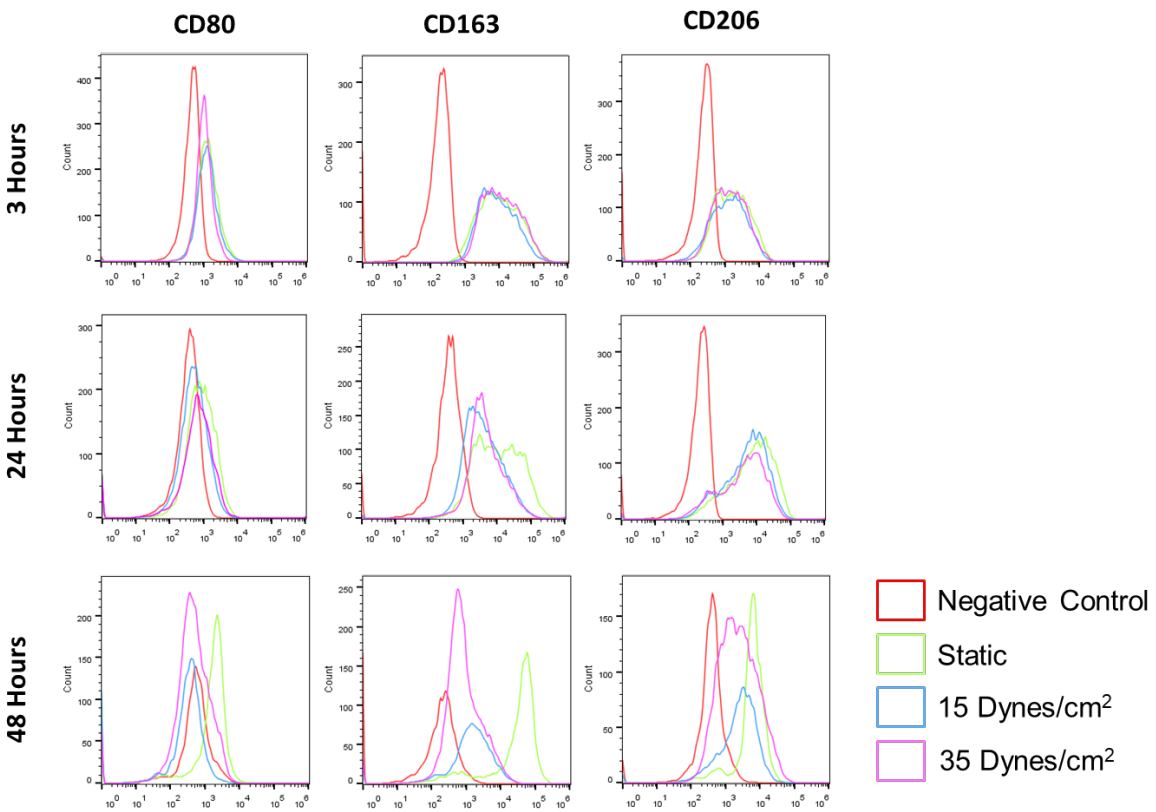

Figure 7. Flow cytometry histograms for CD80, CD163, and CD206 are shown for every timepoint (3, 24, and 48 hours). The data shown are from single samples representative of all data.