Synergistic Effect of Mechanical and Biochemical Stimulation Drives a Pro-Inflammatory Cytokine Profile in Macrophages

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Introduction

Macrophages are a functionally diverse population of innate immune cells that play a key role in maintaining tissue homeostasis. In addition to pathogens and debris, macrophages can also sense a range of dynamic microenvironmental signals, which regulate cell phenotype and function[1]. They are mechanosensitive[2] and respond to mechanical cues in their environment, including the mechanical properties of their mechanical environment[3] and fluid flow-induced shear stress[4].

The foreign body response (FBR) to medical implants poses a major challenge to long-term implant performance, as the resulting cellular reaction disrupts the critical interface between the implant and surrounding tissue, Fig 1. Inflammation is the critical, mediating step of the FBR, transforming initial injury into long-term tissue encapsulation of implants[5]. Our research group has shown that actuation of a mechanotherapeutic implant results in strain and fluid flow at the tissue interface that modulates the FBR and improves therapy delivery[6,7]. Computational models have predicted fluid magnitudes up to 50 mm/s velocity at 1 Hz[7]. In these studies, a significant reduction in the number of neutrophils and a significant reduction in the number of myofibroblasts was observed in the actuated group compared to the static controls[7,8]. Interestingly in these studies we did not observe any significant difference in the number of macrophages present at the timepoints analysed, however the polarisation state of the macrophages was not investigated. In this study we build on these *in vivo* findings and apply a specific fluid shear stress regimen to macrophages *in vitro* and study their resulting behaviour.

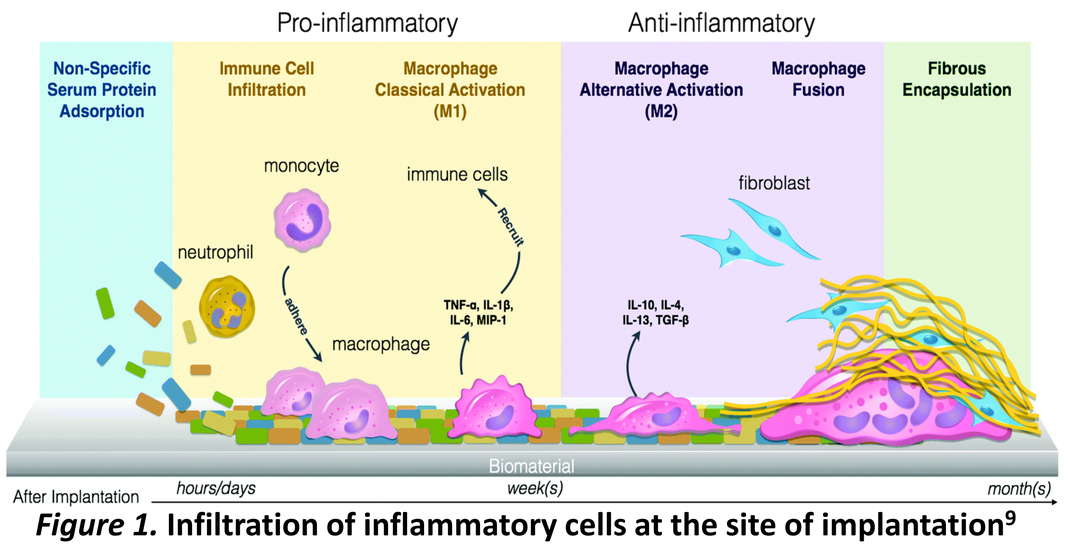


Figure 1. **Infiltration of inflammatory cells at the site of implantation[9]**

Materials and Methods

Bone marrow-derived macrophages (BMDMs) were isolated from C57BL6/J mice (female, 20-25g). Bone marrow from the femur and tibia was extracted into Dulbecco's Modified Eagle Medium (DMEM), red blood cells were lysed, and cells were filtered and resuspended in media. Cells were plated in DMEM containing 20% L929-conditioned media and cultured for 7 days. Cells were harvested, resuspended at 4×10^6 cells/mL, and seeded on Superfrost™ Plus microscopic slides. Three g/different configurations were used to apply either 0.5 or 0.8 Pa fluid shear stress (within the range of computationally predicted peak of ~1.0 Pa, derived from peak fluid velocity of ~50 mm/s[7]), for 10 minutes daily for 2 days, using a parallel plate flow chamber[10] and compared with static controls. Wall shear stress was calculated using the equation for steady, fully developed laminar flow (Equation 1), where Q is the flow rate, μ, the fluid viscosity, b, and h are the width and height of the parallel plate flow chamber[11]. First, naïve macrophages were subjected to the shear stress regimen. Secondly, cells were stimulated with 10 nmL of Lipopolysaccharide (LPS) for 24 h prior to shear stress exposure. Thirdly, we applied the shear stress regimen, and then stimulated the cells with 10 ng/mL LPS for 24 h. In all configurations the supernatants were collected and levels of Tumour growth factor (TNF), Interleukin 6 (IL-6) and Interleukin 10 (IL-10) cytokines were measured using ELISA.

$$\tau_w = \frac{6\,\mu Q}{bh^2} \qquad \text{(Equation 1)}$$

Results and Discussion

Cytokine levels, measured by ELISA, indicated that our shear stress regimen alone did not alter cytokine production (TNF, IL-6, or IL-10) of naïve macrophages, with limited cytokine detection in all groups (Fig. 2a). However, shear stress did alter macrophage cytokine production when LPS was used. Macrophages exposed to LPS prior to our shear stress regimen significantly increased TNF and IL-6 production in the 0.5 Pa group (Fig. 2b), indicating a pro-inflammatory response. When LPS was added after the shear stress regimen, TNF production increased significantly, and IL-10 decreased, suggesting a pro-inflammatory shift compared with static controls (Fig. 2c). Taken together, our results have shown that this shear stress regimen (0.5 or 0.8 Pa at 0.5 Hz) promotes a pro-inflammatory response in BMDMs, but only in synergy with LPS. Fluid shear stress is a potent mechanical stimulus that has been shown to influence macrophage adhesion, morphology, cytokine production, and polarisation, thereby affecting their inflammatory state and functional behaviour[12]. This response is dependent on the magnitude, exposure time, and initial polarisation state of the cells[4]. For instance low magnitude interstitial flow (3 μm/s) polarises macrophages into the pro-healing, alternatively activated phenotype[13], while higher oscillatory shear stress (0 ± 4 dyn/cm² or 0.4 Pa) polarises macrophages to the pro-inflammatory phenotype[14]. Shear stress can synergise with biochemical signals, such as LPS, to activate the NLRP3 inflammasome, escalating immune activation[15]. In the context of the FBR, this work advances our understanding of our previously reported *in vivo* responses[6,7] where we showed that actuation of the implant attenuates the FBR, by identifying that this shear stress regimen promotes the pro-inflammatory response in BMDMs *in vitro*, but only in synergy with LPS.

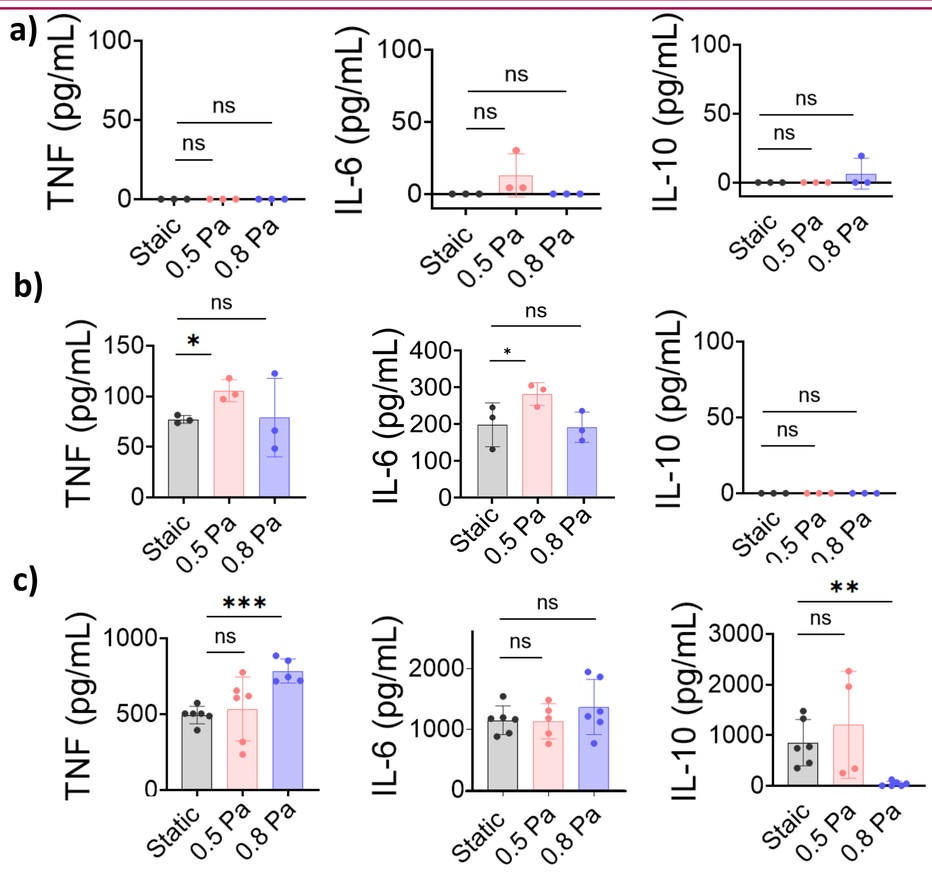


Figure 2. **Shear stress modulation of cytokine levels of naïve macrophages (a), macrophages pre-stimulated with LPS (b), and macrophages stimulated with LPS after fluid flow (c). N=3-6/group**

We hypothesise that mechanical stimulation (actuation of an implant[6,7] or fluid flow-induced shear stress) might accelerate the pro-inflammatory phase and consequently the transition to the pro-healing phase by avoiding chronic inflammation, and promoting a more controlled, temporal macrophage response towards an attenuated FBR; however further studies are required to confirm this.

References: 1. Ward et al., Act Biomat, 2025. 2. Lee et al., Front Cell Dev Biol, 2022. 3. Khanmohammadi et al., Cell Chem Bio, 2025. 4. Jui et al., Ann of Biomed Eng, 2024. 5. Brink et al., Adv Health Mat, 2024. 6. Dolan, E. B. et al. Sci Rob, 2019. 7. Whyte, W. et al., Nat Comm, 2022. 8. Ward et al., Act Biomat, 2024. 9. Kim et al., J Mater Chem B, 2016. 10. Haugh et al., J f Mech Behav of Biomed Mat, 2014. 11. Bacabac et al., J Biomech, 2004. 12. Alarmo et al., Diseases, 2025. 13. Li, R. et al., Mol bio of the cell, 2018. 14. Zhang, S. et al., Heliyon, 2023. 15. Fish et al., ACS App Mats & Interf, 2025.