



Incorporation of Immune Response into Organ-on-a-Chip Technology (CASCADE)

Dylan Fudge, Ph.D.¹, Tyler Goralski, Ph.D.¹, Angela Reinhart², Matthew Gibb, Ph.D.², Vishaka Santosh, Ph.D.¹, Priscilla Lee¹, Desiree Harris¹, Jared Brown, Ph.D.²

¹U.S. Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD ²Skaggs School of Pharmacy, The University of Colorado Anschutz Medical Campus, Aurora, CO



Introduction

A goal for organs-on-chips (OOC) is to recreate living tissues or organ microenvironments with an immune response. The absence of an immune component is a significant drawback to fully recapitulating the physiological human response following exposure to chemical and/or biological agents. Within the innate immune system mast cells are pivotal effector cells contributing to a wide range of downstream immune responses. Mast cells rapidly release a variety of mediators such as histamine, heparin, proinflammatory cytokines & chemokines in response to foreign substances or endogenous damage associated molecular patterns (DAMPs). These factors facilitate immune cell recruitment and play a key role in pulmonary toxicity due to mast cells location within airways and mucosal surfaces of the lungs. The pulmonary system is an ideal exposure route for chemical and biological agents making mast cells a prime choice to assimilate into a lung-on-a-chip. Mast cells are a prime choice to serve as a vital first step in developing OOC technology with an immune component. We incorporated mast cells into the Emulate lung-on-a-chip and exposed to sulfur mustard (HD) to elicit cellular damage and compare to lung-on-a-chip without mast cells. This work allows substantial future work to incorporate other innate immune cells into the system to build towards a complete innate immune system.



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Acknowledgements: R&D funding for CASCADE was provided by DTRA CBS (Sara Peacock & Kristen Fulbright) under project number CB11345. The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense or the U.S. Government.)

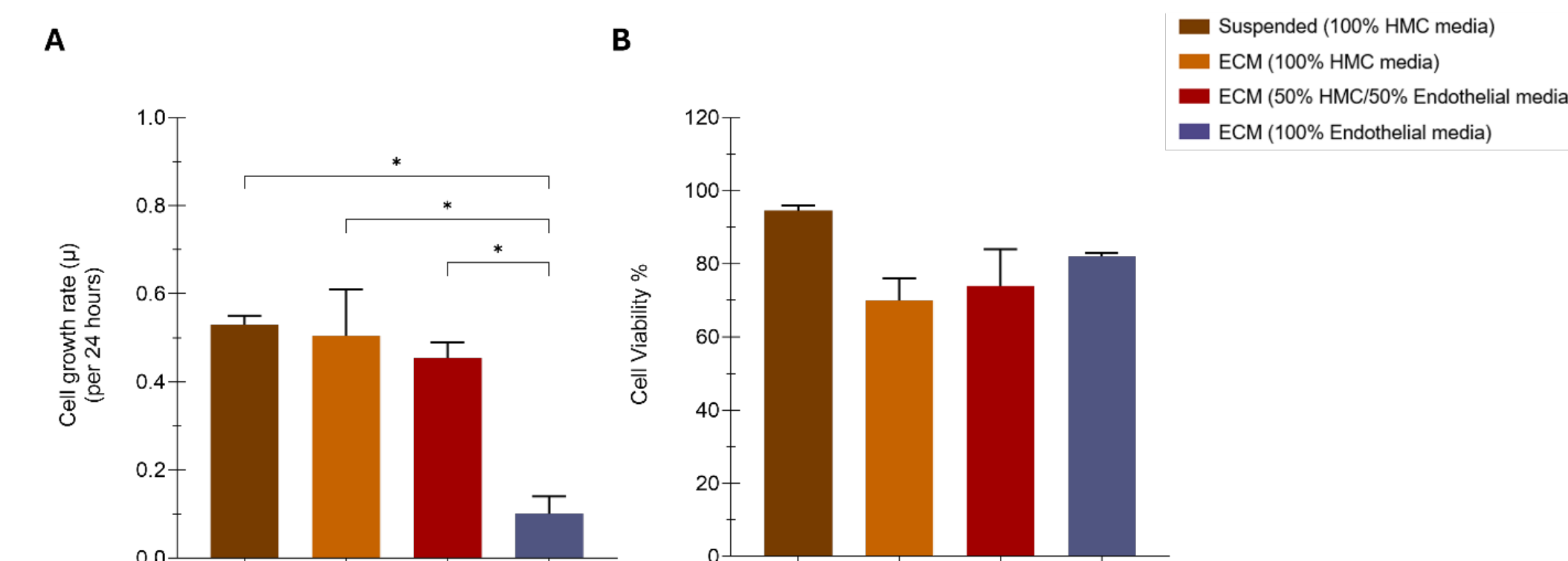


Figure 1: Cell Growth and Viability. Cell growth and viability were measured 48 hours after plating in various media compositions. (A) Cell viability was not significantly changed regardless of media composition. (B) HMC cells showed similar proliferation rates up to 50/50 mix of HMC and Endothelial growth medium, but once in 100% endothelial media, proliferation significantly decreased compared to other media compositions. Data are presented as the mean $\pm$ SEM ($n = 3$); ANOVA with Tukey's *post hoc* test, * $p \leq .05$.

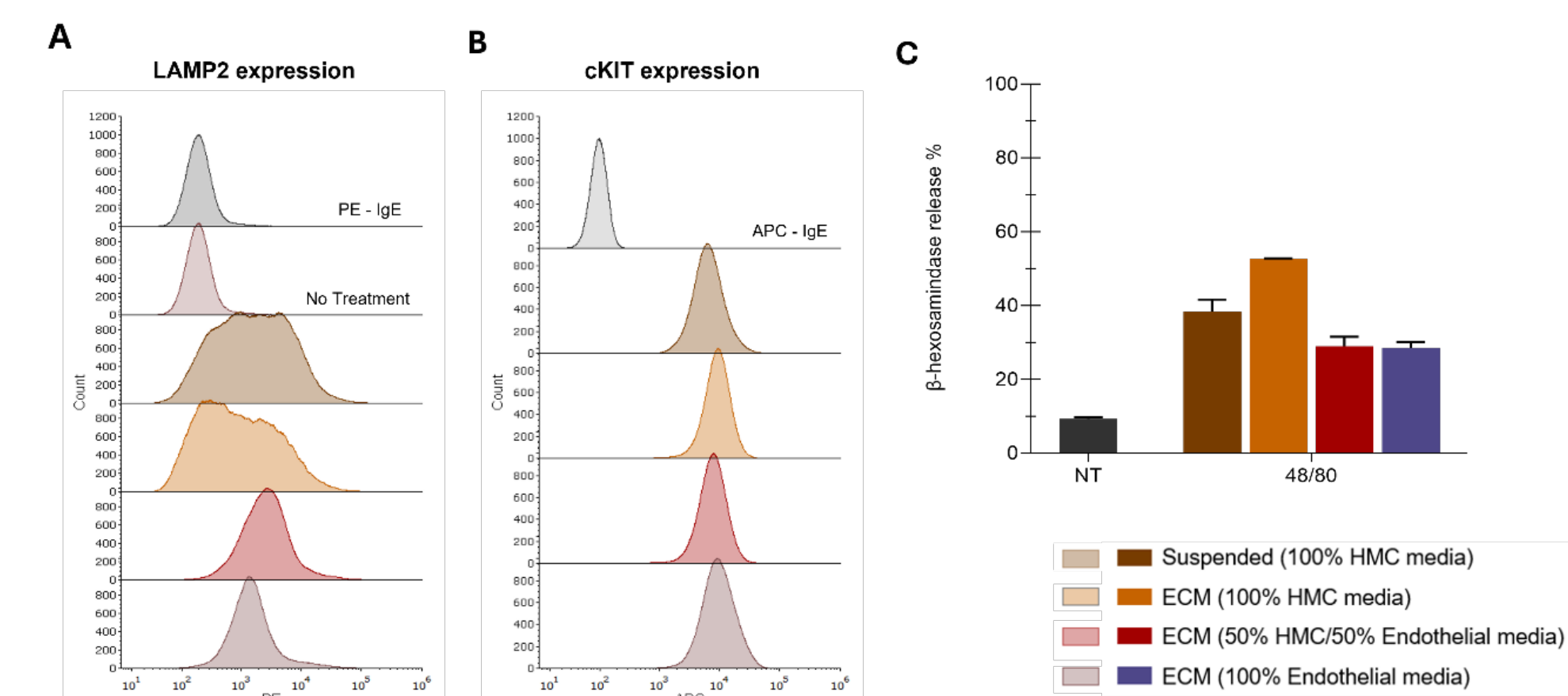


Figure 2: Mast Cell Activation. Degranulation was assessed through the release of (C) β -hexosaminidase (lysosomal enzyme) which was measured in supernatants relative to lysed cells. Activation was assessed by expression of LAMP2 (A) and cKIT (B). At 20 minutes (A) LAMP2 and (B) cKIT expression showed no differences regardless of media composition, with activation noted in all samples. (C) β -hexosaminidase showed that degranulation occurred regardless of media composition, with the most degranulation in HMC media when adhered to ECM. Data are presented as the mean $\pm$ SEM ($n = 3$); ANOVA with Tukey's *post hoc* test, ** $p \leq .01$, **** $p \leq 0.0001$.

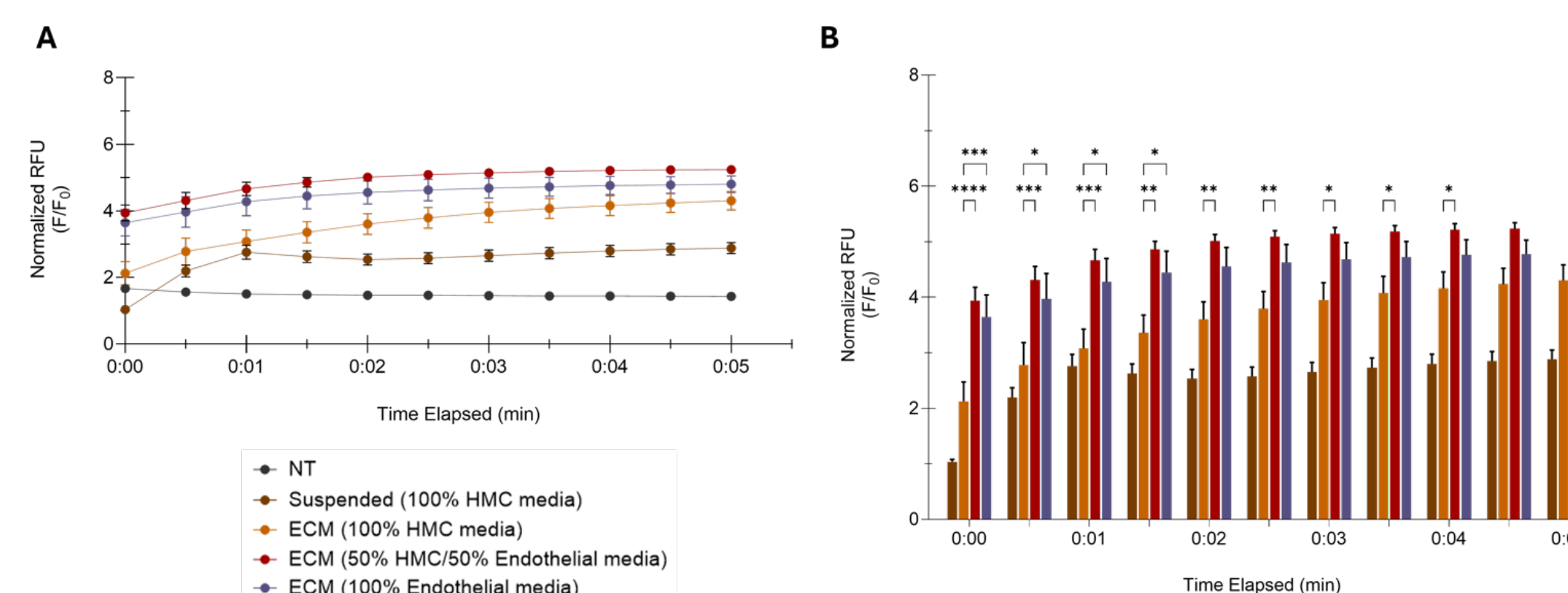
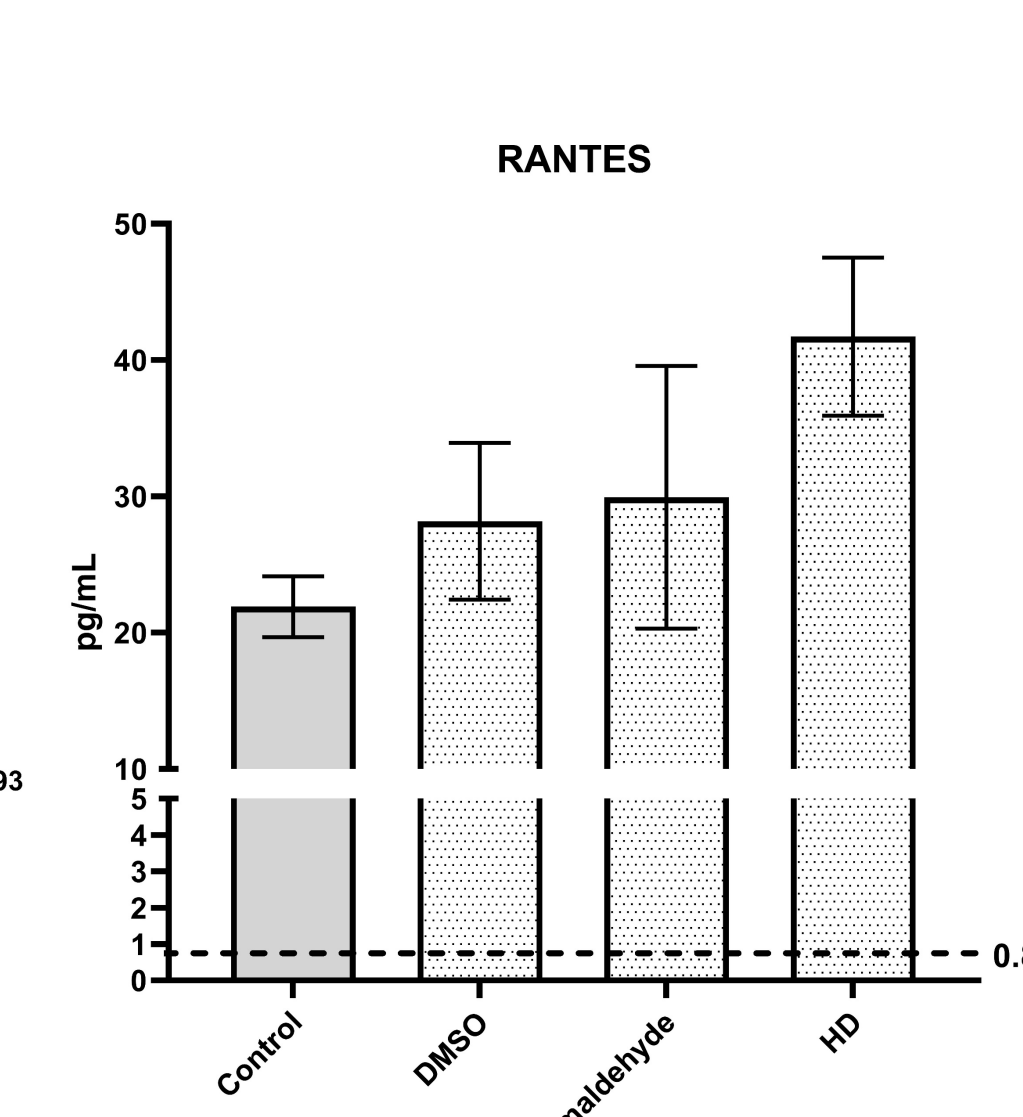
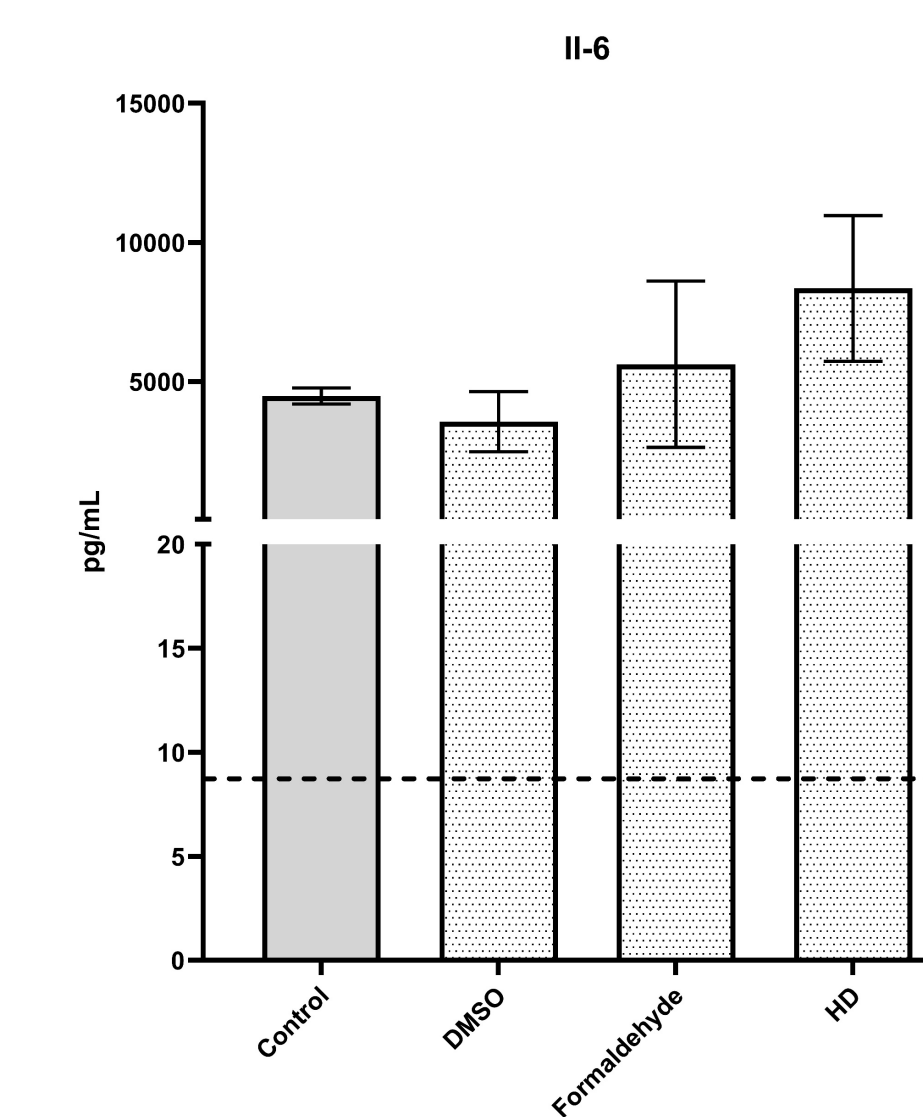
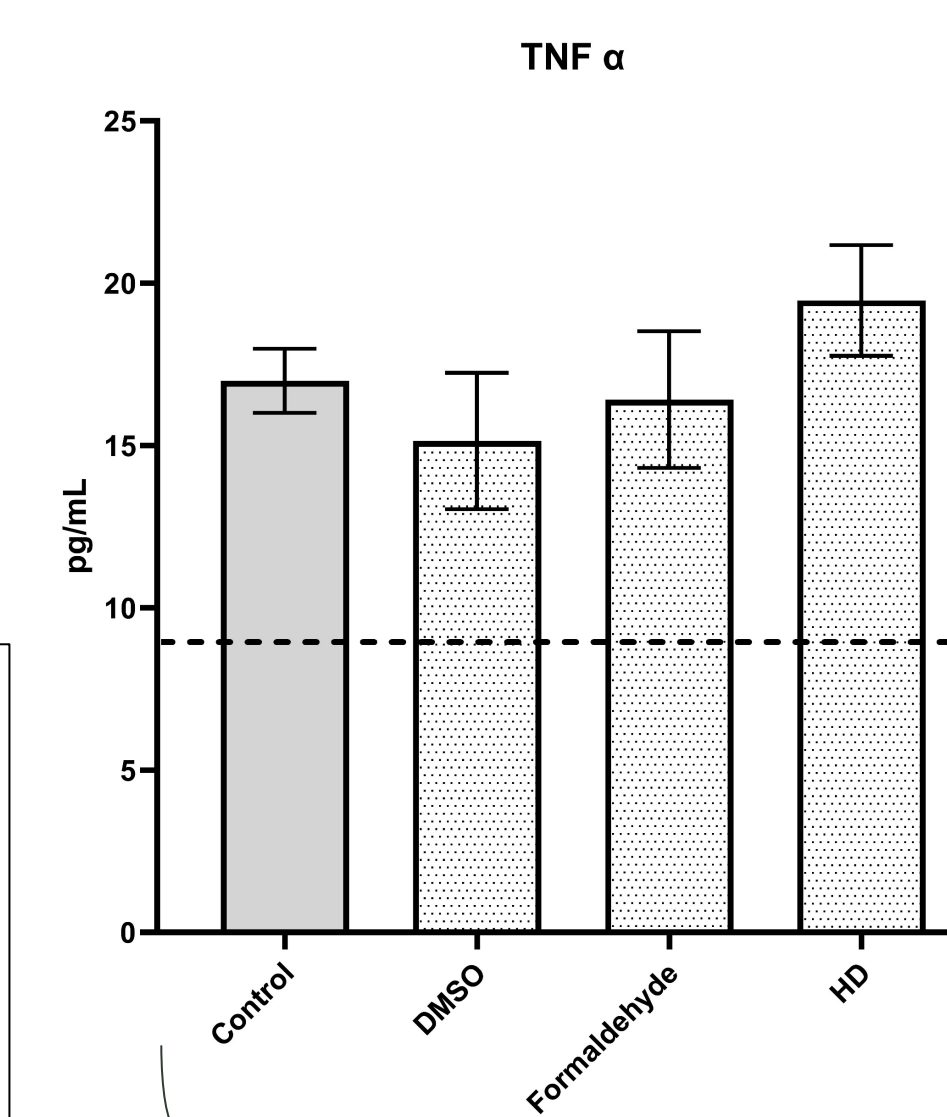
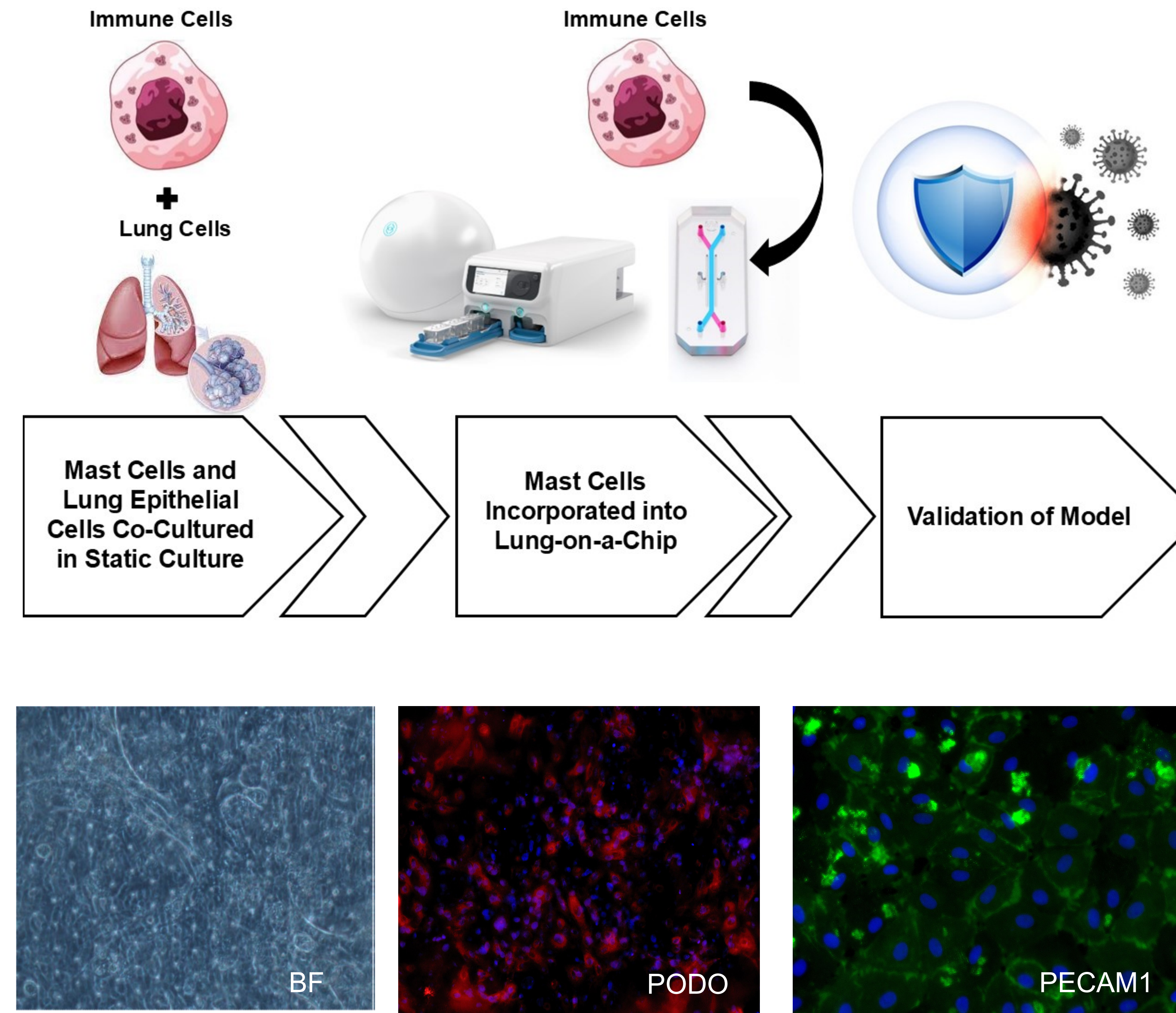
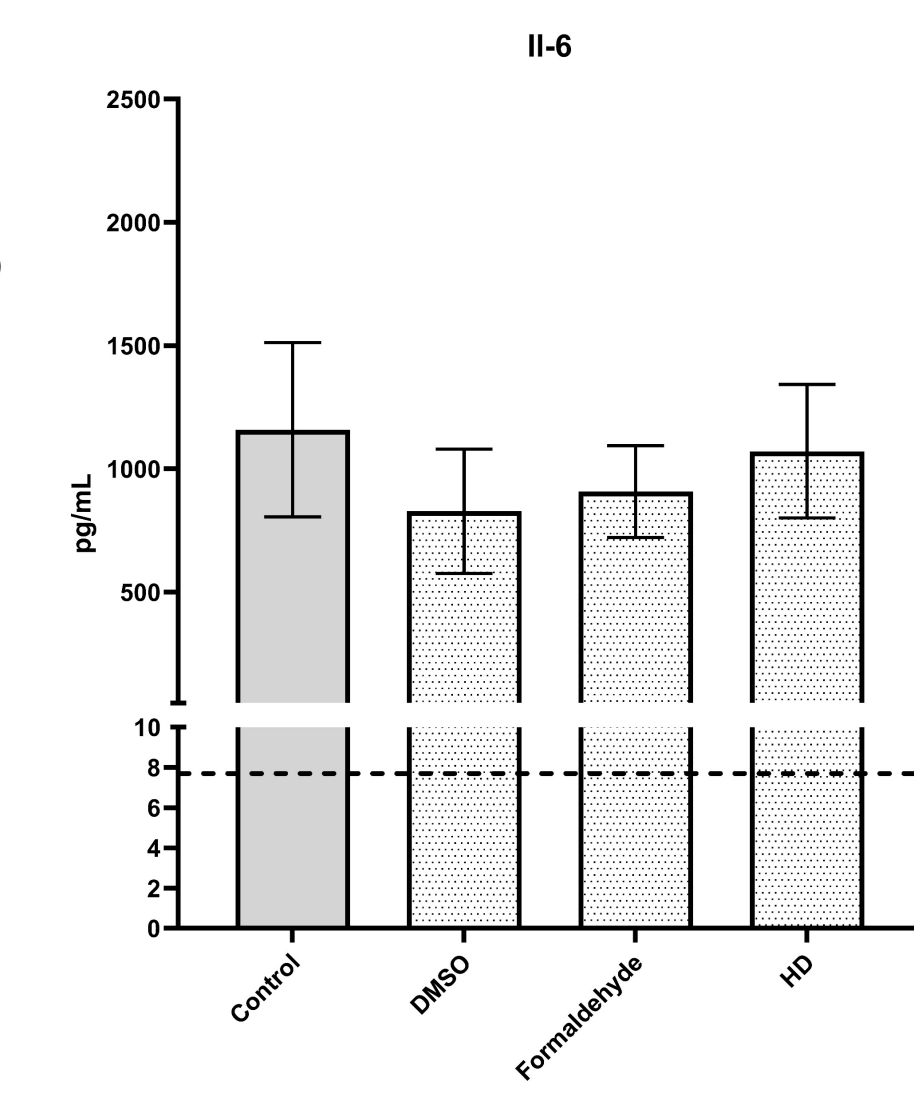
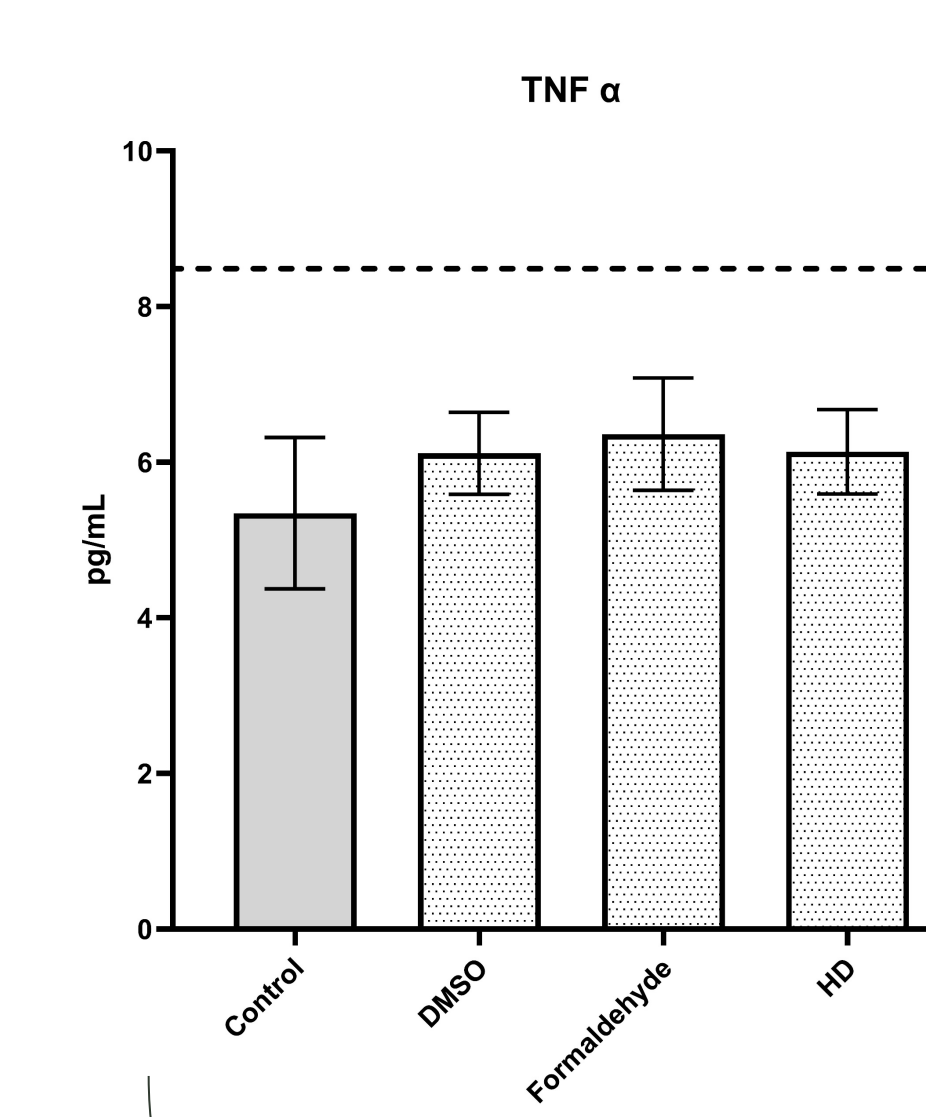


Figure 3: Quantitative analyses of the relative Ca^{2+} , Ca^{2+} levels after stimulation by 48/80. Trends in Ca^{2+} flux were similar regardless of media composition through 5 minutes (A). Differences can be seen in basal levels of Ca^{2+} after 48/80 exposure based on media composition chosen (B). Data are presented as the mean $\pm$ SEM ($n = 3$); ANOVA with Tukey's *post hoc* test, * $p \leq 0.05$, ** $p \leq .01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.



Endothelial Channel with Mast Cells



Endothelial Channel without Mast Cells