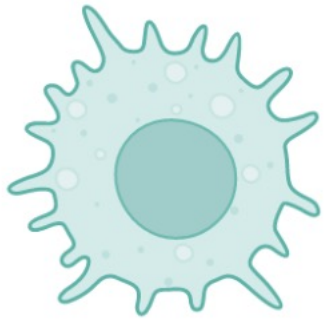
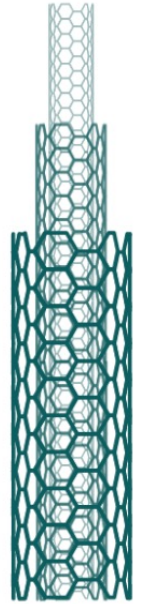
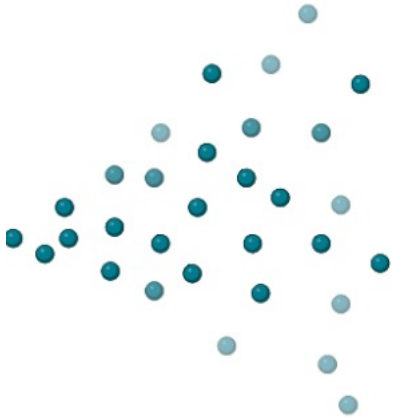




# Immunomodulatory Effects of Multi-Walled Carbon Nanotubes on THP-1 Macrophages



**Tanvi Nandikonda**

**Supervisor: Marjo Petäjäaho**

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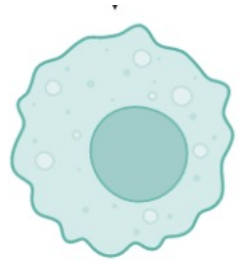
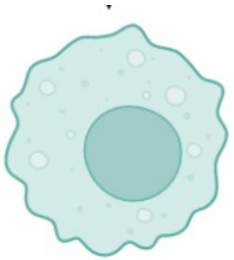
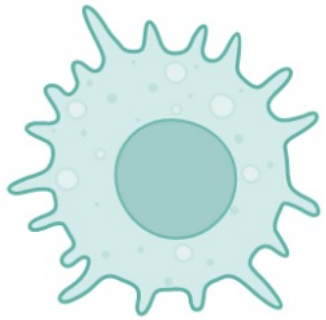
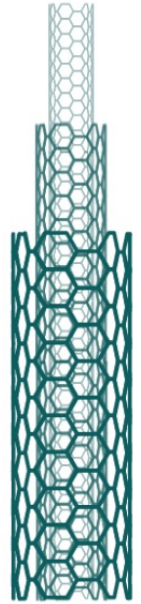


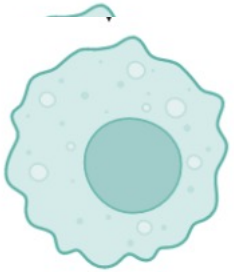


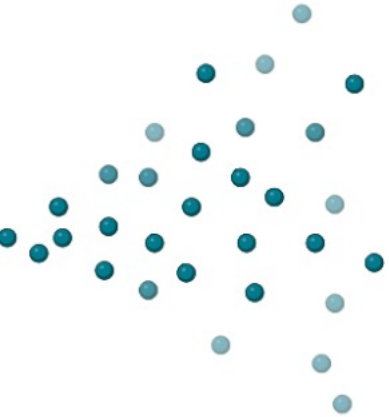


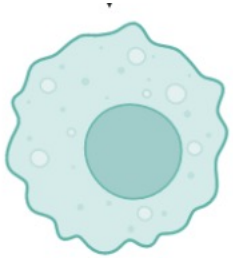


## Research Question

How do varying concentrations of multi-walled carbon nanotubes (3.0, 6.5, 15.0 µg $mL^{-1}$) affect the gene expression of selected cytokine genes (IL1B, IL4, IL6, CXCL8, IL10, IL11, IL12p35, IL12p40, IL13, IL18, IFNG, TGFB1, TNF) in THP- 1 macrophages from *Homo sapiens*?







# 1. Purpose and Significance of Investigation

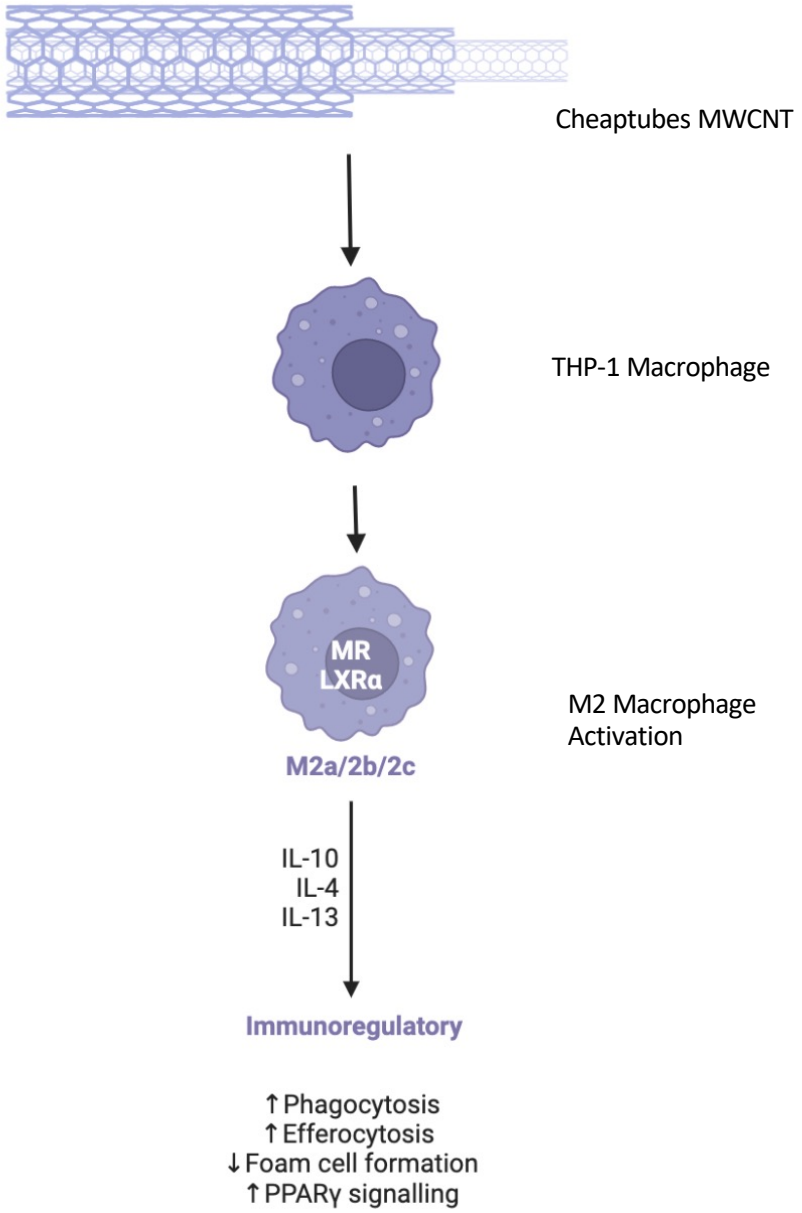
- Multi-walled carbon nanotubes (MWCNTs) are a category of engineered nanomaterials (ENMs) which have gained widespread commercial interest due to their notable physicochemical properties.
- However, the rapid growth of MWCNT utilization raises concerns about its toxicity in occupational health, with previous studies concluding that exposure to MWCNTs induces asbestos-like pathogenicity and lung inflammation in *in vivo* murine models.
- Ergo, there is an acute requirement for the effective characterization of this nano-bio interaction to develop appropriate safety protocols and therapeutics.
- This knowledge can then be used to evaluate toxicity and provide a foundation to manufacture engineered nanomaterials that could be safe and functional by design.

# 2. Background Information

- Macrophages are white blood cells which maintain homeostasis in immune system by eliminating cell debris and foreign matter and communicating with other cells through antigen display and cytokine secretion.
- Cytokines are cell-signalling proteins which mediate inter-cellular communication to generate appropriate response against endogenous abnormalities (ex. mitigate local inflammation)
  - Proinflammatory Cytokines (IL-8, IL-12, TNF, IL-6, IFN- γ, IL-18, IL-1β): promote inflammation by increasing production of other proinflammatory cytokines → differentiate macrophages into classically activated M1 phenotype
  - Anti-inflammatory Cytokines (IL-4, IL-13, IL-10, TGF-B, IL-11): suppress production of pro-inflammatory cytokines → avert M1 differentiation + promote alternatively activated M2 differentiation
- Macrophages attempt to remove MWCNTs from tissue via phagocytosis → if clearance is unsuccessful, macrophages initiate inflammatory reaction by producing pro-inflammatory cytokines to recruit other immune cells.
- The nano-bio interactions between MWCNTs and macrophages is largely dependent on the physicochemical properties of MWCNTs (such as size, shape, and chemical composition).

# 3. Hypothesis

- There is limited information about the effects of Cheaptubes on THP-1 transcriptomic response, so gene expression trends for Bayertubes (MWCNT with similar dimensions to Cheaptubes) from Kinaret et al. 2020 is used to predict outcome for Cheaptubes.
- Assumption and Framework: MWCNTs with similar physical properties exhibit similar trends in macrophage transcriptomic response.
- At low cytotoxic concentrations of Bayertubes, Kinaret et al. 2020 study reveals differential downregulation of proinflammatory genes TNF and IL1B as the MWCNT concentration increases.
- This reveals suppression of pro-inflammatory genes, it can be inferred that Bayertubes showcases evidence of **anti-inflammatory M2 macrophage activation**, which *I predict to observe for Cheaptubes as well in this investigation*.



# 4. Materials and Methodology

## Variables

- **Independent Variable:** MWCNT Concentration
- **Dependent Variable:** Cytokine Gene Expression
- **Controlled Variables:**
  - Cell Type: THP-1 cell line (*Homo sapiens*)
  - MWCNT Brand: Cheaptubes
  - Time of Exposure: 24 hours

## Materials

- **Statistical Analysis:** Microsoft Excel Version 2021
  - Data Analysis ToolPak
- **Presentation of Graphs:** GraphPad Prism
- **Raw Data:** Transcriptomic Data from GrecoLab, Tampere University

# 4. Materials and Methodology

## Methodology

- This is a data-based investigation conducted in collaboration with Tampere University and the University of Helsinki
- How did I obtain my data? (The following steps were performed by GrecoLab research group)
  - **WST-1 cytotoxicity assay**: determine the $IC_5$, $IC_{10}$, and $IC_{20}$ concentrations of Cheaptubes for the THP-1 cell line.
  - **DNA microarray apparatus**: characterise transcriptional alterations after 24 hours of Cheaptubes MWCNT exposure for the three low-cytotoxic concentrations (3.0, 6.5, 12.0 $\mu g\ mL^{-1}$)
  - **Pre-Processing**: quality check, probe filtering, normalization, batch estimation and correction, and annotation → generate $Log_2$-transformed pre-processed data
- How did I process my data?
  - The log-transformed DNA microarray data obtained from their case study was used as input for further processing and interpretation using statistical techniques.
    - **$Log_2$ Fold Change** calculated in comparison to control group for each gene to determine change in gene expression
    - **Single-Factor ANOVA** used to assess statistical significance of change in gene expression between treatment groups
    - If $p < 0.05$ → resulting data is subjected to **Tukey-Kramer post-hoc test** to determine which two groups exhibit a statistically significant difference

# 5. Results

## Experimental Results

- Differential Expression threshold: $Log_2FC = \pm 0.58$
  - **3.0 µg mL$^{-1}$**: no genes
  - **6.5 µg mL$^{-1}$**: IL1B and TNF (downregulation)
  - **15.0 µg mL$^{-1}$**: IL1B and TNF (downregulation)
- **Proinflammatory Cytokine Genes:** Cheaptubes MWCNT exposure downregulated the expression of the proinflammatory cytokine genes (with exception to IL18).
  - Furthermore, IL1B and TNF display evidence of dose-dependent downregulation as the concentration of Cheaptubes MWCNT increases from 3.0 to 15.0 µg mL$^{-1}$.
- **Anti-Inflammatory Cytokine Genes:** The exposure to low cytotoxic concentrations of Cheaptubes induced downregulation of IL11 and no statistically significant changes for TGBF1 and IL10.
- INFG, IL4, IL13, IL12p35, and IL12p40 mRNA were not identified nor collected by the microarray apparatus.
- These results are compatible with the trends observed in Bayertubes from the Kinaret et al. study, indicating that Bayertubes displays potential of a valid model to predict cytokine gene expression and macrophage differentiation in Cheaptubes. Hence, this supports my hypothesis regarding the potential paralleled transcriptomic response between the two MWCNTs which have similar physical properties. (SUMMARISE)

# 5. Results

## Graphical Representations (IL1B and TNF)

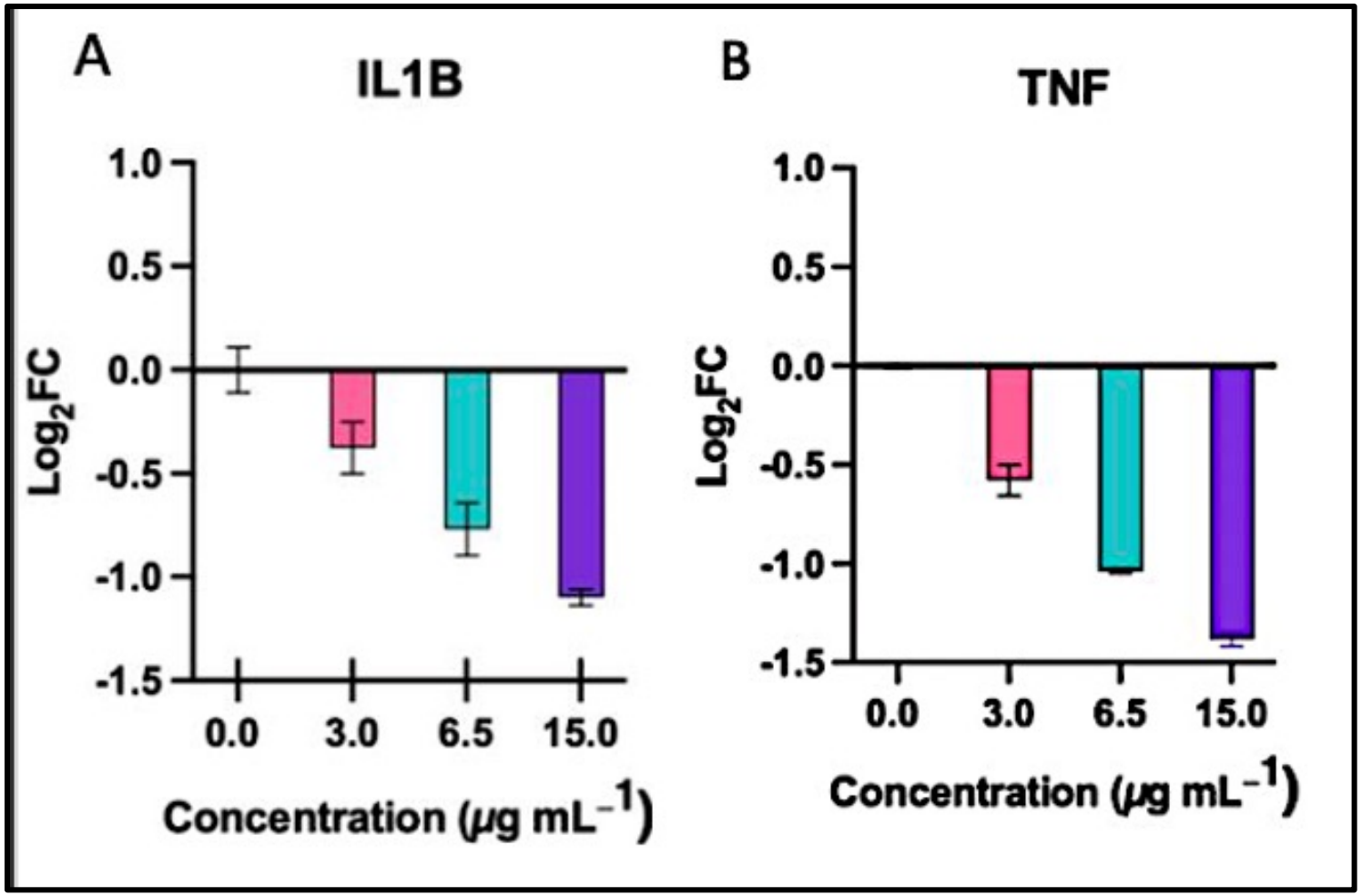



*Figure 1: Gene expression levels ($Log_2$ Fold Change) of IL1B (A), TNF (B) with Cheaptubes (MWCNT) concentrations at 3, 6.5, 15 μg $mL^{-1}$ at 24 hr post- exposure compared to untreated control (0.0 μg $mL^{-1}$)*

# 5. Results

## Graphical Representations (Other Proinflammatory Cytokine Genes)

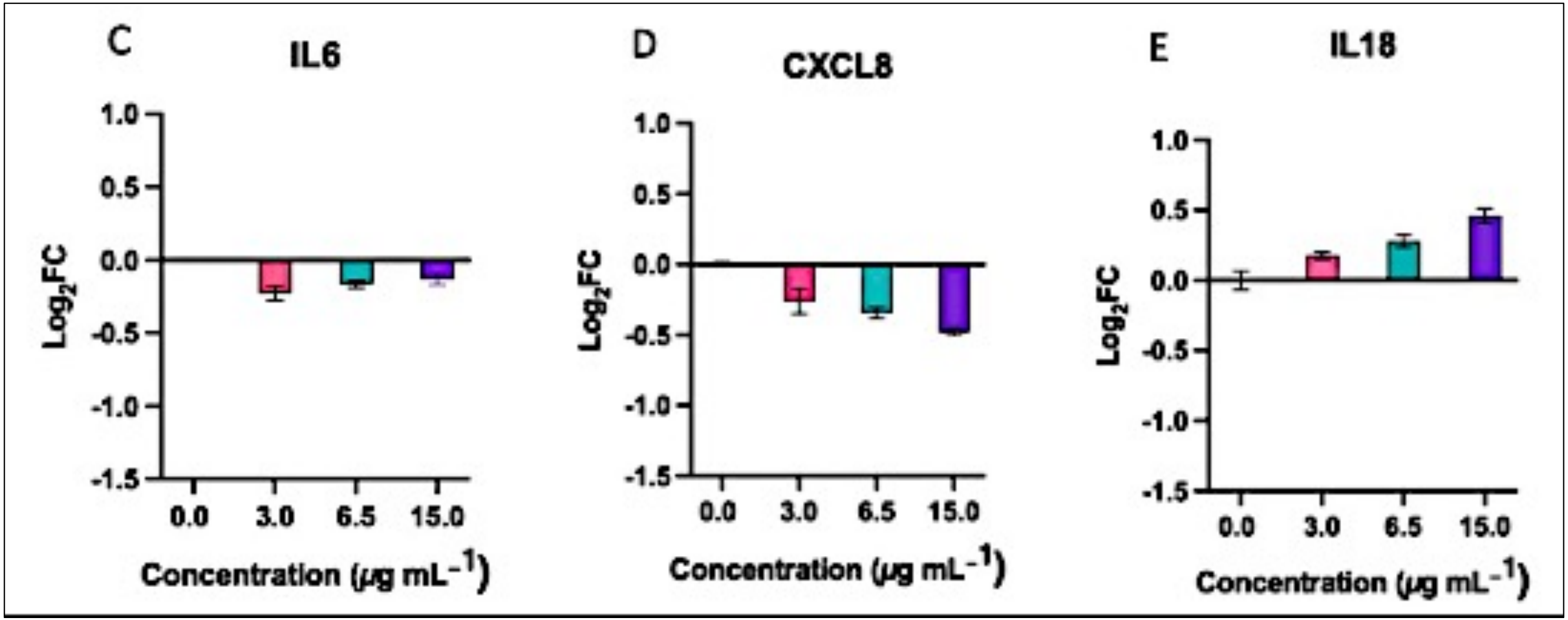



*Figure 2: Gene expression levels (Log2 Fold Change) of IL6 (C), CXCL8 (D), IL18 (E) with Cheaptubes (MWCNT) concentrations at 3, 6.5, 15 µg mL−1 at 24 hr post- exposure compared to untreated control (0.0 µg mL−1)*

# 5. Results

## Graphical Representations (Anti-Inflammatory Genes)

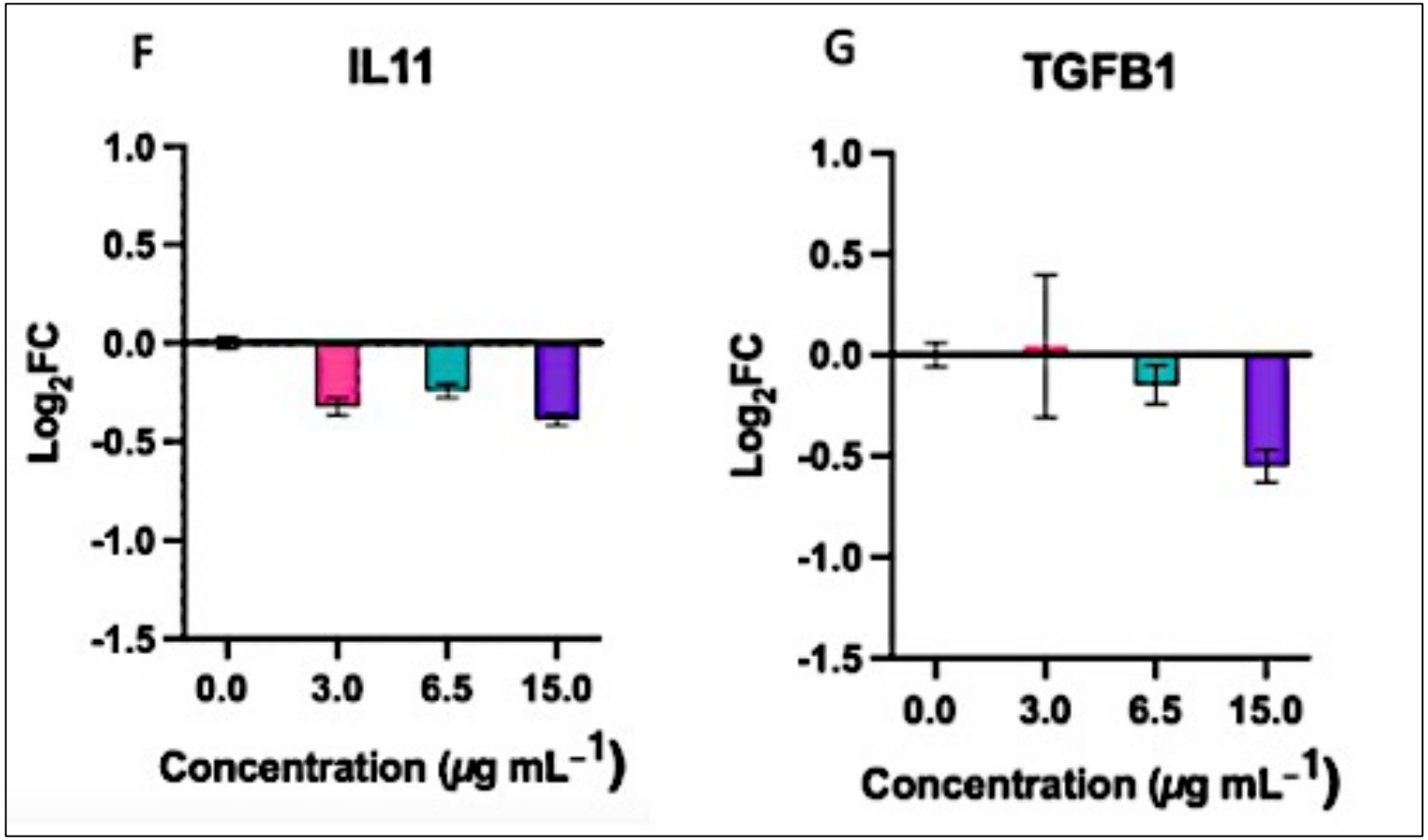



*Figure 3: Gene expression levels ($Log_2$ Fold Change) of IL11(F), TGFB1 (G) with Cheaptubes (MWCNT) concentrations at 3, 6.5, 15 μg $mL^{-1}$ at 24 hr post-exposure compared to untreated control (0.0 μg $mL^{-1}$)*

# 6. Conclusion

- For low cytotoxic concentrations, it can be concluded that Cheaptubes MWCNT induce alternative M2 macrophage specialization in THP-1 macrophages within the given scope and limitations of the experiment.
- This is because differential gene expression results convey that THP-1 macrophages suppress the main M1 pro-inflammatory genes TNF and IL1B.

# 7. Literature Review

- The aforementioned conclusion can be supported by results from past studies regarding the effects of MWCNT diameter on immune cell response.
  - Rydman et al. 2013:
    - MWCNTs with diameter ≥ 50 nm → proinflammatory and genotoxic response
    - MWCNTs with diameter between 8 nm and 15 nm did not produce any significant responses.
  - Kinaret et al. 2020:
    - rCNT rigid carbon nanotubes (diameter = 50 nm) and GNF graphite nano fibres (diameter = 140 nm) → proinflammatory response with upregulation and release of TNF and IL1B
    - Cheaptubes and Bayertubes (diameter < 20 nm) → downregulation of the proinflammatory cytokine genes TNF and IL1B
- Hence, the diameter or thickness of multi-walled carbon nanotubes (MWCNT) may affect the macrophage response.
  - Carbon nanomaterials with a diameter greater than 50 nm → upregulation of pro-inflammatory cytokines → M1 macrophage activation
  - Carbon nanomaterials with a diameter less than 20 nm → downregulation of pro-inflammatory cytokines → M2 macrophage activation
- However, it is important to acknowledge that individual MWCNT properties cannot fully explain transcriptomic response due to synergistic interplay between different features affecting macrophage activation. In the future, more studies concerning the effect of shape and size on macrophage response should be effectuated to validate the aforementioned reasoning and conclusions.

# 8. References


- Illustrations on PowerPoint Presentation: BioRender (https://www.biorender.com/)
- Graphical Representations: GraphPad Prism (https://www.graphpad.com/features)
- Studies Referenced in Presentation:
  - Kinaret, Pia Anneli, et al. “Carbon Nanomaterials Promote M1/M2 Macrophage Activation.” *Small*, vol. 16, no. 21, 2020, p. 1907609., https://doi.org/10.1002/smll.201907609.
  - Rydman, Elina et al. “Evaluation of the Health Effects of Carbon Nanotubes.” *Final Report on Project Number 109137 of the Finnish Work Environment Fund.*, 2013, https://www.julkari.fi/handle/10024/135079.