

Cellular Expression of Inflammatory Mediators Upon Exposure to Invisalign Elute: An In-vitro Study

2024 Research Aid Awards (RAA)

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FollowUp Form

Award Information



In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Title of Project:*

Cellular Expression of Inflammatory Mediators Upon Exposure to Invisalign Elute: An In-vitro Study

Award Type

Research Aid Award (RAA)

Period of AAOF Support

July 1, 2024 through June 30, 2025

Institution

Nova Southeastern University

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)

Xiaozhe Han, Thyagaseely Premaraj, Elaheh Dalir Abdolahinia

Amount of Funding

\$6,000.00

Abstract

(add specific directions for each type here)

file uploaded

Respond to the following questions:

Detailed results and inferences:*

If the work has been published, please attach a pdf of manuscript below by clicking "Upload a file".

OR

Use the text box below to describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should also be attached by clicking "Upload a file".

Follow up Results.pdf

Exposure to aligner elute reduced OBA-9 cells viability and caused a significant increase in IL-1 β and decrease in IL-1Ra gene expression.

Components from clear aligners containing multilayer aromatic thermoplastic polyurethane may trigger innate inflammatory responses by the oral epithelial barrier.

This disruption of immune balance could be associated with the IL-1 signaling pathway and the activation of NF- κ B.

These findings may bring more mechanistic insights into potential aligner-associated hypersensitivity reactions, which will help clinicians for the early identification and prevention of more severe complications.

Were the original, specific aims of the proposal realized?*

Yes, the original hypothesis: the exposure to aligner elute would trigger an imbalanced release of pro-inflammatory and anti-inflammatory mediators in oral epithelial cells (OBA-9) was supported. We observed an increase in IL-1 β (a pro-inflammatory mediator) and a decrease in IL-1Ra (an anti-inflammatory mediator).

Were the results published?*

No

Have the results of this proposal been presented?*

Yes

To what extent have you used, or how do you intend to use, AAOF funding to further your career?*

AAOF funding has played and will continue to play a pivotal role in advancing my academic and professional career in orthodontics. It has enabled me to conduct meaningful research on the inflammatory and hypersensitivity responses of oral epithelial cells to aligner materials, contributing to both scientific knowledge and clinical practice.

All funds were fully utilized to support data collection, analysis, and presentation of findings. This experience has been instrumental in my development as a clinician-scientist and will continue to support my goal of publishing impactful research and promoting evidence-based orthodontic care.

Accounting: Were there any leftover funds?

\$0.00

Not Published

Are there plans to publish? If not, why not?*

We have not yet published our results, as data collection for the THP-1 cells is still ongoing. Additionally, we are investigating the discrepancy between IL-1Ra expression at the RNA level and its absence at the protein level. We plan to publish our findings once a clear and consistent pattern emerges across both the THP-1 and OBA-9 cell lines.

Presented

Please list titles, author or co-authors of these presentation/s, year and locations:*

Expression of Inflammatory Mediators in Oral Epithelial Cells (OBA-9) Upon Exposure to Aligner Elute: An In-vitro Study. co-authors - Thimanthi Withana, Elaheh Abdolahinia, Thyagaseely Premaraj, Xiaozhe Han. E-Poster presented at 2025 AAO annual conference in Philadelphia.

Was AAOF support acknowledged?

If so, please describe:

yes

Internal Review

Reviewer comments

Reviewer Status*

File Attachment Summary

Applicant File Uploads

- Follow up Results.pdf

Results

MTT

In our MTT experiment, one-way ANOVA was conducted by comparing each group to the 2% saliva negative control, and the findings are summarized in Figure 1. No statistical difference was observed between media and 2% saliva. 1% MDI showed significant cytotoxicity across all time points ($p < 0.0001$), while 0.5% MDI was not statistically significant. The 2% 1-week elute showed a significant decrease in cell viability at 24h ($p < 0.001$), whereas the 2% 4-week elute was not significant at this time point. However, at 48h and 72h, both 2% 1- and 4-week elutes exhibited significantly decreased viability ($p < 0.0001$). No cytotoxicity was observed for 1% and 0.5% elutes at 24h, although significant decreases were noted at 48h and 72h for both 1- and 4-week elutes.

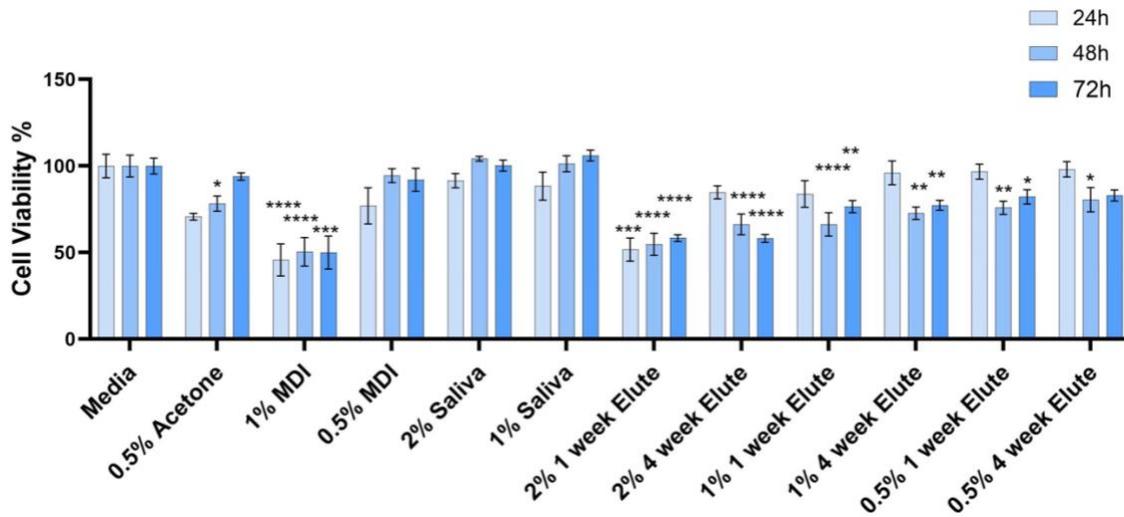


Figure 1. MTT Experiment: OBA-9 cell viability after exposure to 1- and 4-week elutes at 0.5%, 1%, and 2% dilutions for 24-72h. One-way ANOVA vs. 2% saliva. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Live/Dead Fluorescence

The Live/Dead dual staining kit was used to assess the cellular and membrane integrity of OBA-9 cells following exposure to various treatments. Live cells were identified by the presence of Calcein AM, which produced an intense green fluorescence (excitation/emission ~494 nm/~517 nm), while dead cells were distinguished by Ethidium homodimer-1, which produced a bright red fluorescence (excitation/emission ~494 nm/~617 nm). The proportion of live and dead cells was quantified based on fluorescence intensity, using manufacturer-recommended methods.

Figures 2–4 present Live/Dead staining images of OBA-9 cells following 24h, 48h, and 72h of exposure to various controls and elutes. Figures E–F, representing 1% and 2% saliva, a slight recovery in cell viability is observed over time, as indicated by an increase in green fluorescence from 24h to 72h. In contrast, Figures I–J show a pronounced increase in red fluorescence with MDI exposure, confirming its cytotoxicity effect.

Figures C–D, G–H, and K–L depict the effects of 1-week and 4-week elutes at various concentrations. A concentration-dependent decrease in green fluorescence is observed, with higher concentrations of elute correlating with fewer live cell staining. Across all samples, green fluorescence was consistently higher at 24h compared to 48h and 72h, suggesting a time-dependent reduction in cell viability with prolonged exposure. Notably, cytotoxicity appeared to be more pronounced for the 1-week elutes compared to the 4-week elutes.

Based on the results of MTT Experiment and Live/Dead staining, 2% concentrations of both the 1-week and 4-week elutes were found to be optimal—maintaining sufficient cell viability while still being capable of eliciting an inflammatory response. As a result, 2% 1-week and 4-week elutes, along with 2% saliva (negative control) and 1% MDI (positive control), were selected for use in subsequent experiments.

Additionally, while 24h, 48h, and 72h timepoints were initially tested during the viability screening phase to determine optimal exposure durations, the results showed no substantial differences between 48h and 72h. Therefore, to reduce redundancy and optimize resource use, only the 24h and 72h timepoints were selected for future experiments.

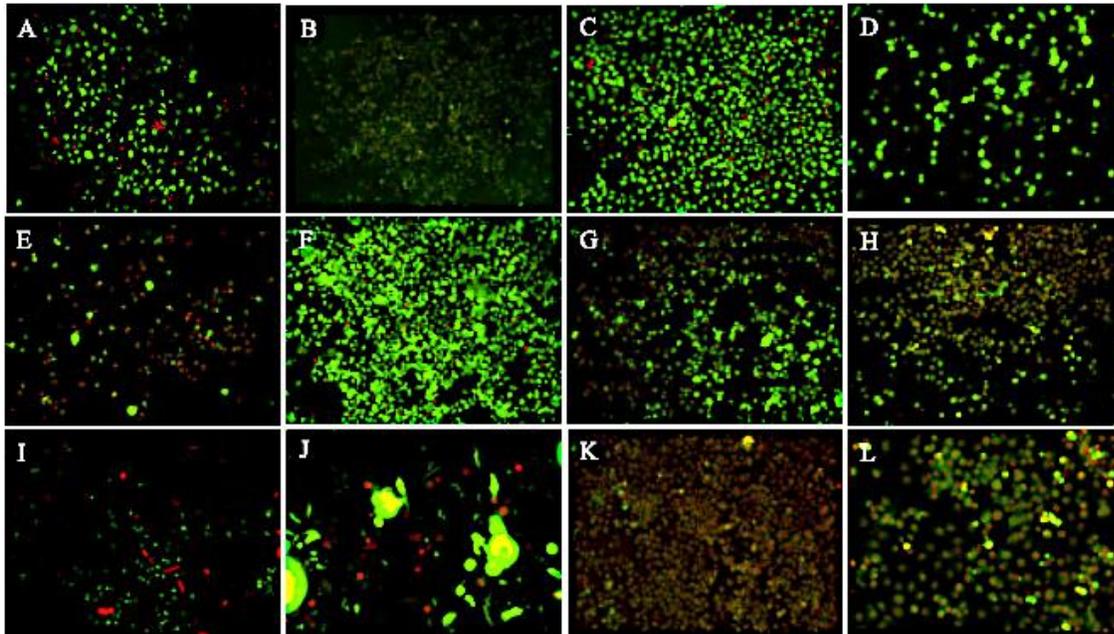


Figure 2. 24h Live/ Dead staining of OBA-9 (4x). (A) Media; (B) 0.5% Acetone; (E-F) 1% and 2% Saliva; (I-J) 0.5% and 1% MDI/ Acetone; (C, G, K) 0.5%, 1% and 2% 1-week elute; (D, H, L) 0.5%, 1% and 2% 4-week elute.

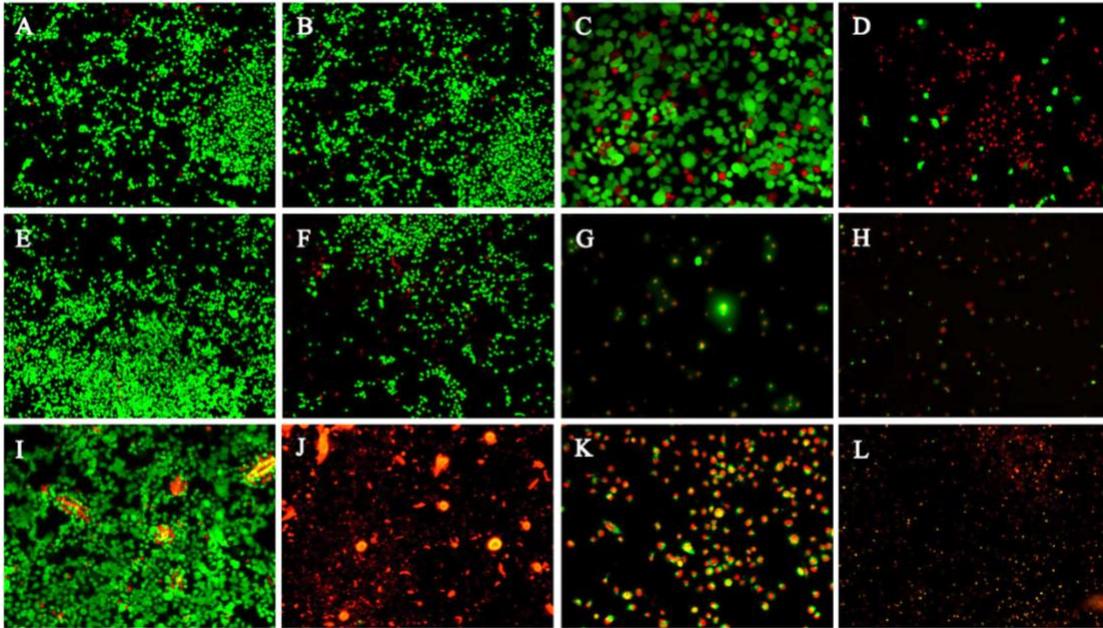


Figure 3. 48h Live/ Dead staining of OBA-9 (4x). (A) Media; (B) 0.5% Acetone; (E-F) 1% and 2% Saliva; (I-J) 0.5% and 1% MDI/ Acetone; (C, G, K) 0.5%, 1% and 2% 1-week elute; (D, H, L) 0.5%, 1% and 2% 4-week elute.

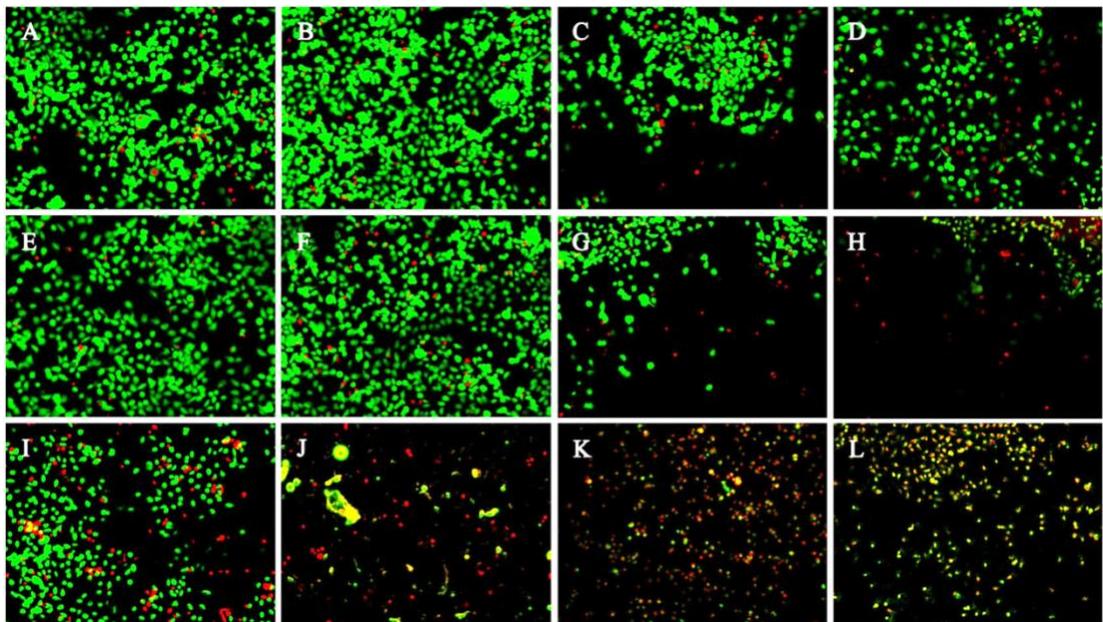


Figure 4. 72h Live/ Dead staining of OBA-9 (4x). (A) Media; (B) 0.5% Acetone; (E-F) 1% and 2% Saliva; (I-J) 0.5% and 1% MDI/ Acetone; (C, G, K) 0.5%, 1% and 2% 1-week elute; (D, H, L) 0.5%, 1% and 2% 4-week elute.

Real Time Quantitative Polymerase Chain Reaction (RT-qPCR)

RT-qPCR analysis was performed using primers for IL-1 β , IL-8, ICAM-1, IL-1Ra, TGF- β , PDGF-BB, TNF- α , IL-13, IL-17A, with GAPDH as the internal control. Gene expression was assessed in OBA-9 cells exposed to 1-week and 4-week elutes at 24h and 72h, with results summarized in Figures 5–10. IL-8, PDGF-BB, TGF- β , and ICAM-1 were expressed, but no statistical significance was observed. TNF- α , IL-13, and IL-17A showed no expression in OBA-9 cells.

Notably, exposure to the 1-week elute resulted in a statistically significant upregulation of IL-1 β expression at 72h ($p < 0.05$), suggesting a pro-inflammatory response after prolonged exposure. Additionally, the 4-week elute demonstrated a statistically significant downregulation of IL-1Ra at 72h ($p < 0.01$), indicating a potential impairment in anti-inflammatory regulatory mechanisms over time.

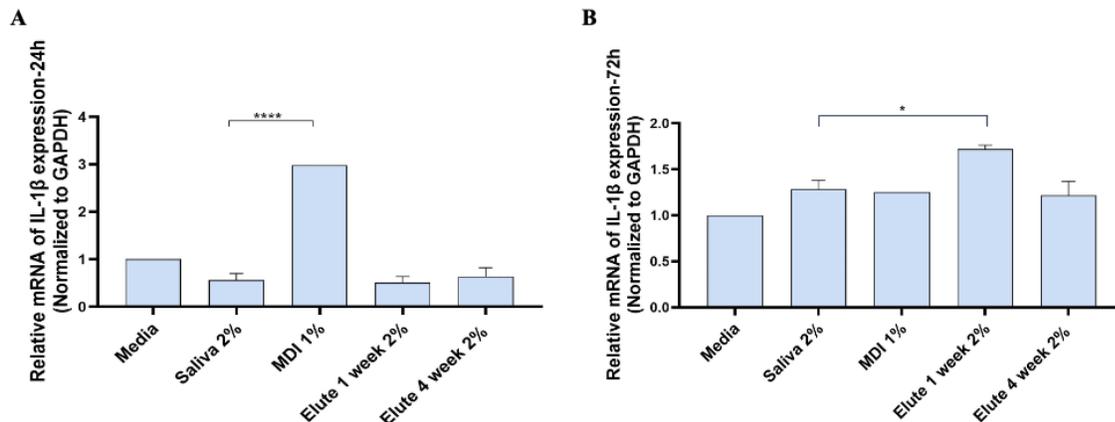


Figure 5. Gene expression of IL-1 β relative to GAPDH in OBA-9 upon exposure to 1 and 4 week elutes at (A) 24h and (B) 72h.

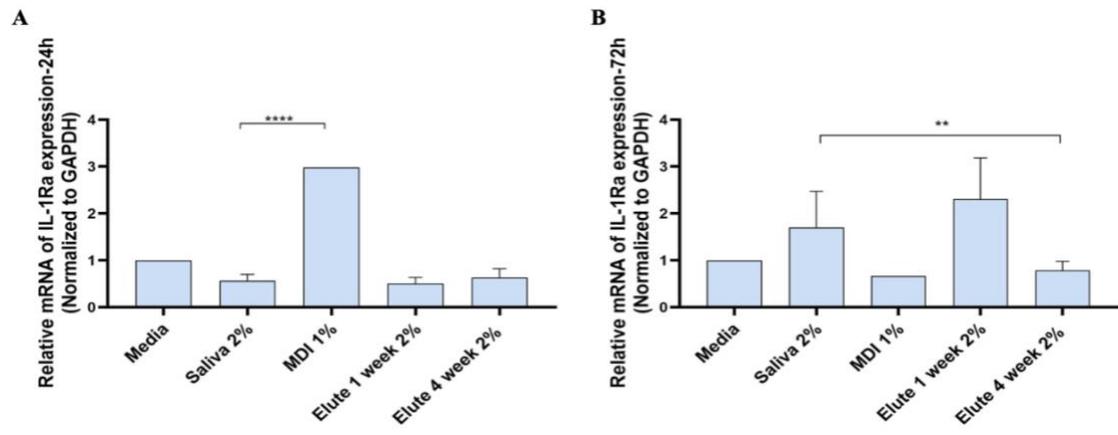


Figure 6. Gene expression of IL-1Ra relative to GAPDH in OBA-9 upon exposure to 1 and 4 week elutes at (A) 24h and (B) 72h.

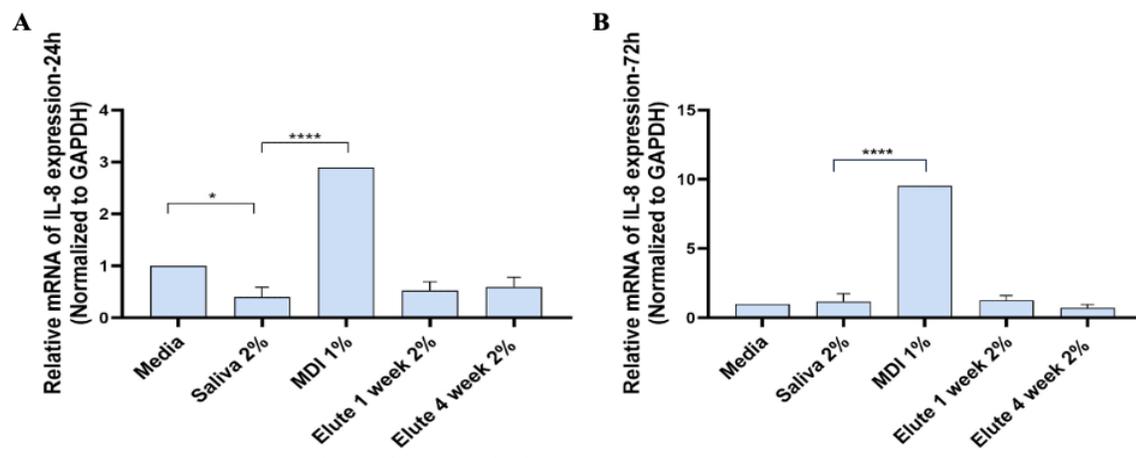


Figure 7. Gene expression of IL-8 relative to GAPDH in OBA-9 upon exposure to 1 and 4 week elutes at (A) 24h and (B) 72h.

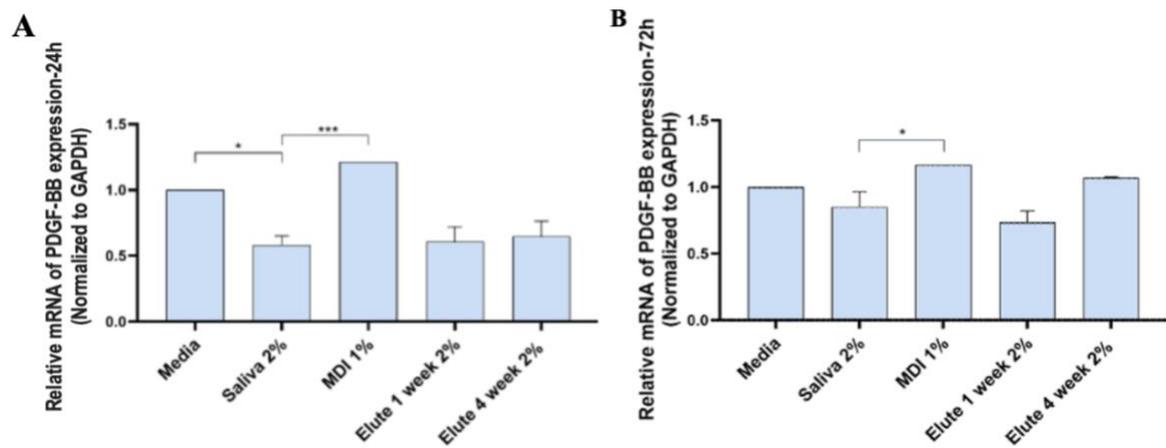


Figure 8. Gene expression of PDGF-BB Relative to GAPDH in OBA-9 upon exposure to 1 and 4 week elutes at (A) 24h and (B) 72h.

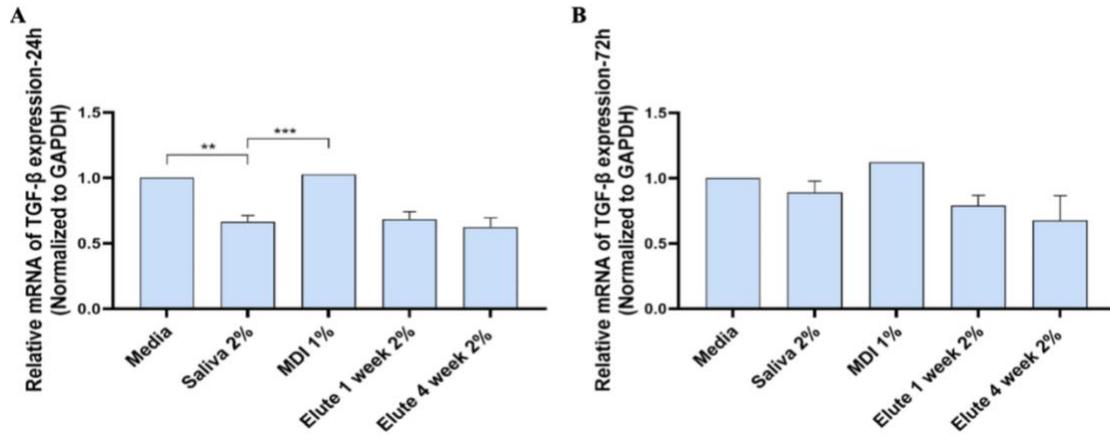


Figure 9. Gene expression of TGF- β relative to GAPDH in OBA-9 upon exposure to 1 and 4 week elutes at (A) 24h and (B) 72h.

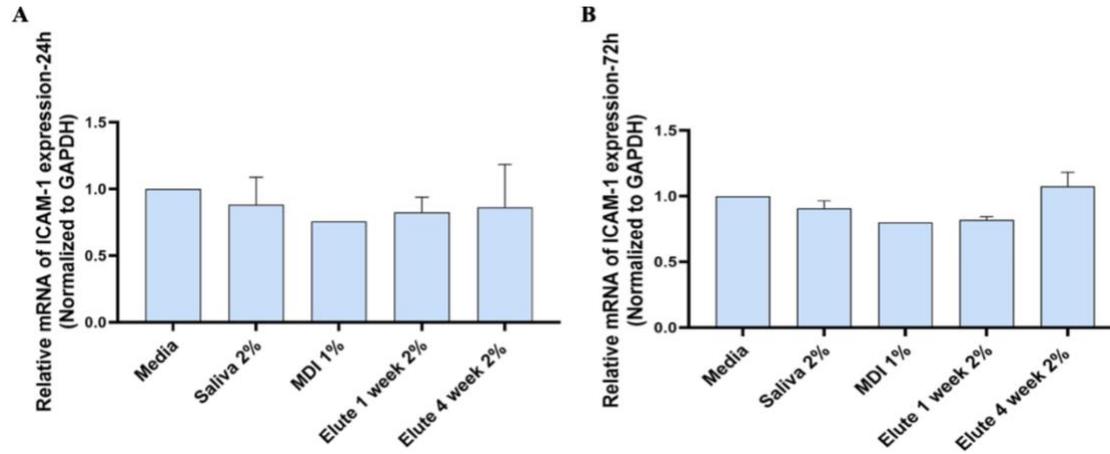


Figure 10. Gene expression of ICAM-1 relative to GAPDH in OBA-9 upon exposure to 1 and 4 week elutes at (A) 24h and (B) 72h.

Enzyme-Linked Immunosorbent Assay (ELISA)

Since IL-1 β and IL-1Ra demonstrated significant changes in gene expression, these cytokines were further analyzed at the protein level using enzyme-linked immunosorbent assay (ELISA). OD values were measured at a wavelength of 450 nm using a microplate reader to quantify protein expression. While IL-1Ra expression was undetected, IL-1 β expression is shown in Figure 11. Both the 1-week and 4-week elutes exhibited significantly increased IL-1 β protein levels at 24h ($p < 0.01$ and $p < 0.001$, respectively). However, no significant IL-1 β protein expression was seen at 72h.

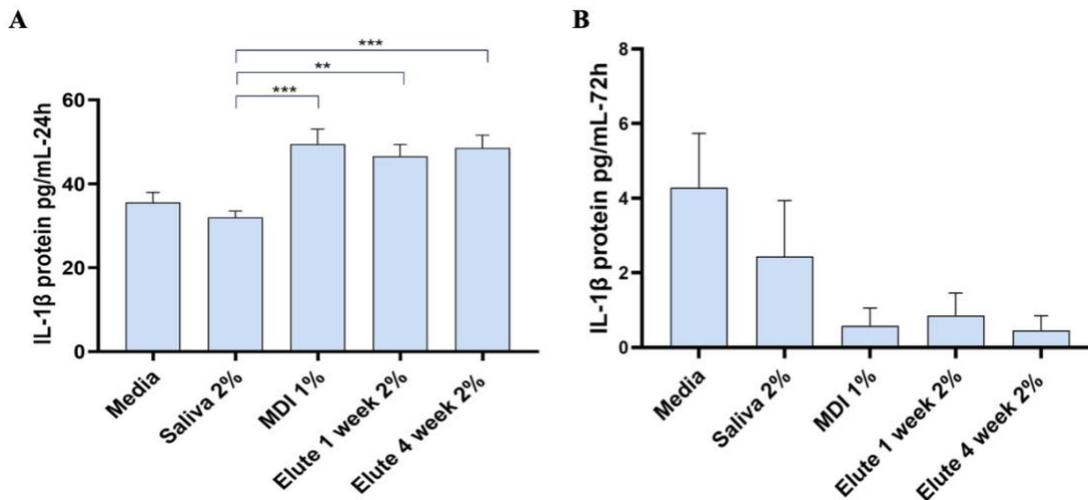


Figure 11. Protein expression of IL-1 β in OBA-9 upon exposure to 1- and 4- week elutes at (A) 24h and (B) 72h.