

# Toxicity and Inflammatory Response to Common Magic Mouthwashes in a 3D Oral Reconstructed Tissue Model

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## Introduction

Magic Mouthwash is a general term describing oral rinses prescribed by a doctor for the treatment of pain, inflammation, or infection, commonly as a result of chemotherapy and radiation induced oral sores (oral mucositis). There is no set combination of ingredients for the preparation of a magic mouthwash, rather they are formulated for the individual needs of each patient, selecting from an array of various active ingredients. Although the ingredients most commonly used to formulate Magic Mouthwashes are considered safe as prescribed, the final formulations lack toxicity and inflammatory response data. Four common Magic Mouthwash formulations (MM 1,2,3,4), which vary slightly in active and inactive ingredients, were tested for cytotoxicity and inflammatory response using a novel testing strategy based on a commercially available (3D) EpiOral™ tissue model (MatTek Corporation, Ashland, MA), reconstructed from normal human-derived oral epithelial cells. This *in vitro* model can be used to accurately and reproducibly assess the safety of products designed to treat oral mucosal conditions and thus reduce the burden of clinical studies.

## Materials & Methods

### Magic Mouthwashes Contain Aqueous Solutions

- Sweeteners (MM 1,2,3,4)
- Antihistamine (MM 1,2,3,4)
- Anesthetic (MM 1,3,4)
- Anti-inflammatory (MM 2,3,4)
- Antifungal (MM 2,4)
- Antacid (MM 1)

### Duplicate MatTek EpiOral™ 3D Tissues

- 4 Magic Mouthwashes (MM 1,2,3,4)
- Negative Control (NC), Water (30 min., 24 hr.)
- Positive Control (PC), 1% TritonX-100 (20 min., 1 hr., 2 hr.)

### Clinical Exposure Reflected by Topical Application

- 5 min. MM exposures represent time spent swishing
- 30 min. MM exposures imitate clinical exposure before rinsing
- 16 hr. MM exposures mimic a full day of exposure

### ELISA Immune Response Assay

- Media collected to quantify cytokines secreted
- IL-1β measured compared to standard (16 hr. MM and 30 min. NC)

### MTT Cytotoxicity Assay

- Extracted formazan salt (purple) indicates cell survival
- Cytotoxicity generally correlates with irritancy
- Provides % Cell Survival and potentially ET<sub>50</sub> values
- Two-way (cytotoxicity) or one-way (immune response) ANOVA used, \* p < 0.05

## Results

### Magic Mouthwash 1 (MM 1)

- AA: Mylanta (33%)
  - Al(OH)<sub>3</sub> (8%)
  - Mg(OH)<sub>2</sub> (8%)
  - Simethicone (0.8%)
- AN: Lidocaine (0.7%)
- AH: Diphenhydramine (0.1%)

### Magic Mouthwash 3 (MM 3)

- AN: Lidocaine (0.7%)
- AH: Diphenhydramine (0.08%)
- AI: Dexamethasone (0.004%)

### AA: Antacid

AF: Antifungal

AH: Antihistamine

AI: Anti-inflammatory

AN: Anesthetic

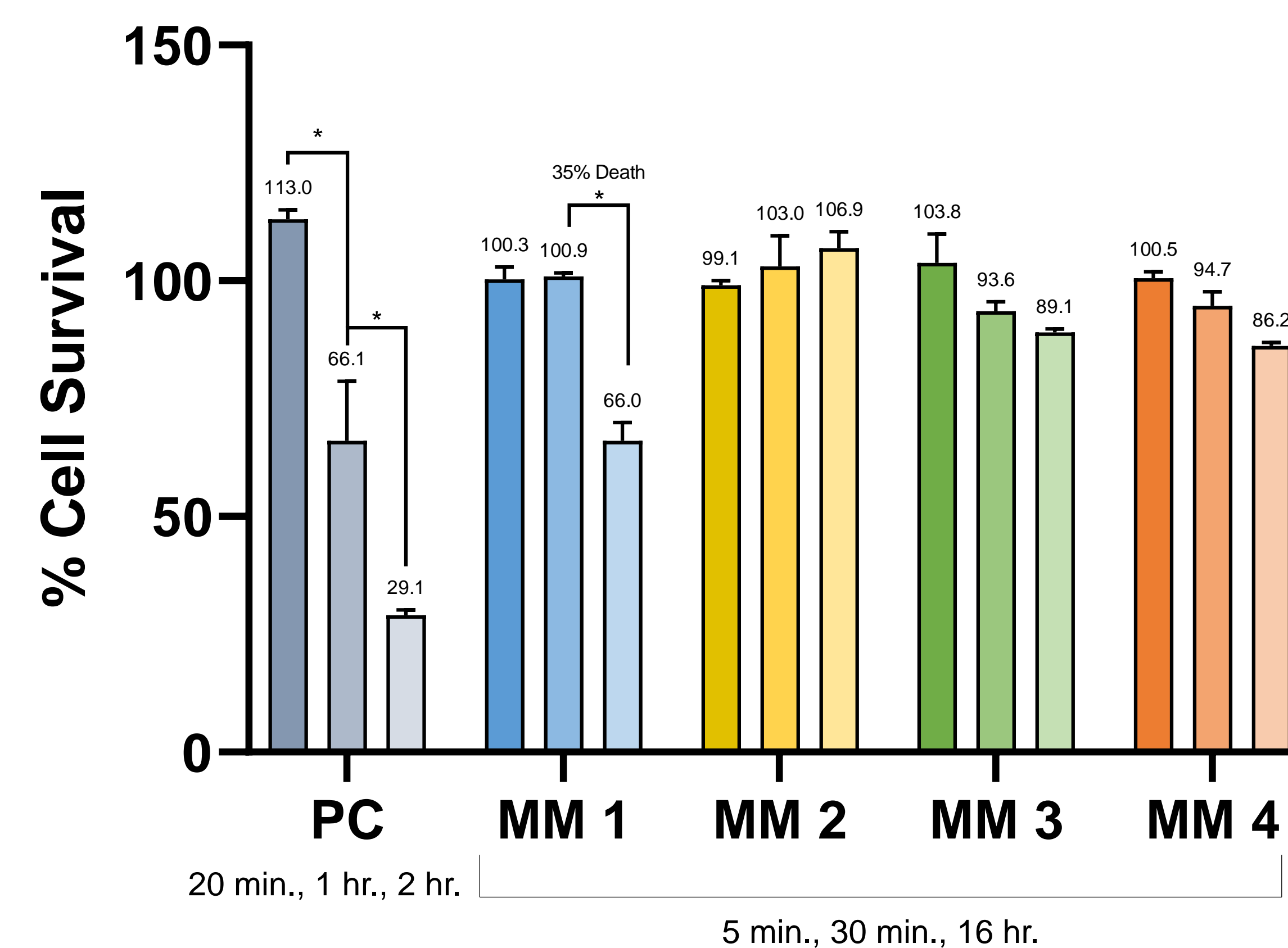
### Magic Mouthwash 2 (MM 2)

- AI: Hydrocortisone (0.1%)
- AF: Nystatin (0.08%)
- AH: Diphenhydramine (0.025%)

### Magic Mouthwash 4 (MM 4)

- AN: Lidocaine (0.5%)
- AF: Nystatin (0.4%)
- AI: Prednisolone (0.075%)
- AH: Diphenhydramine (0.0625%)

## Magic Mouthwash Cytotoxicity

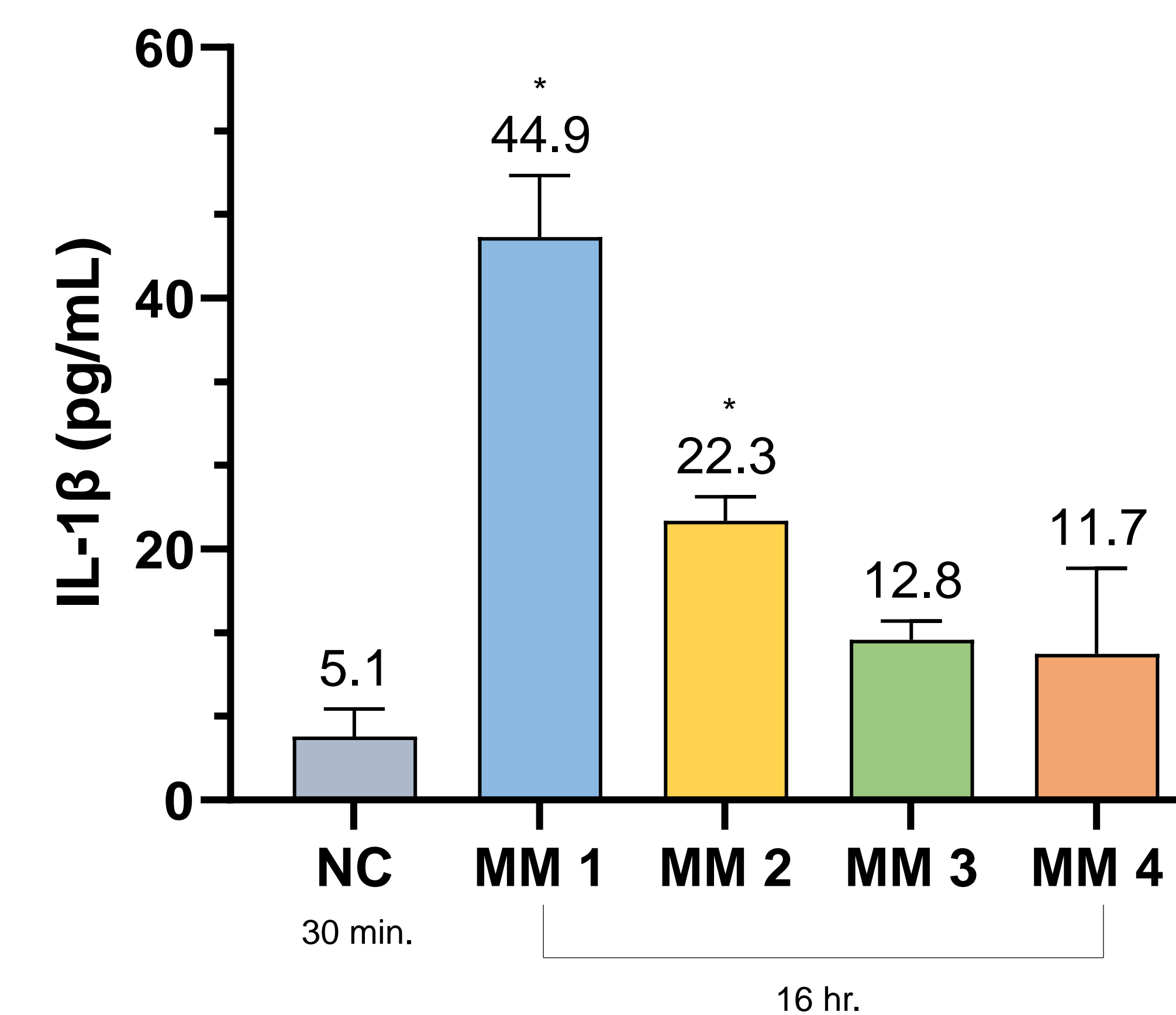


**Figure 1.** Cytotoxicity data (MTT Assay) demonstrate differences between the Magic Mouthwash formulations. MM 1 showed a significant difference in cell viability between the short-term exposures and the 16 hour exposure (35% cell death). MM 2 was the only mouthwash that was not observed to induce cell death over time.

Magic Mouthwash 1  
35% Cell Death

Magic Mouthwash 2  
No Cell Death

## Magic Mouthwash Immune Response



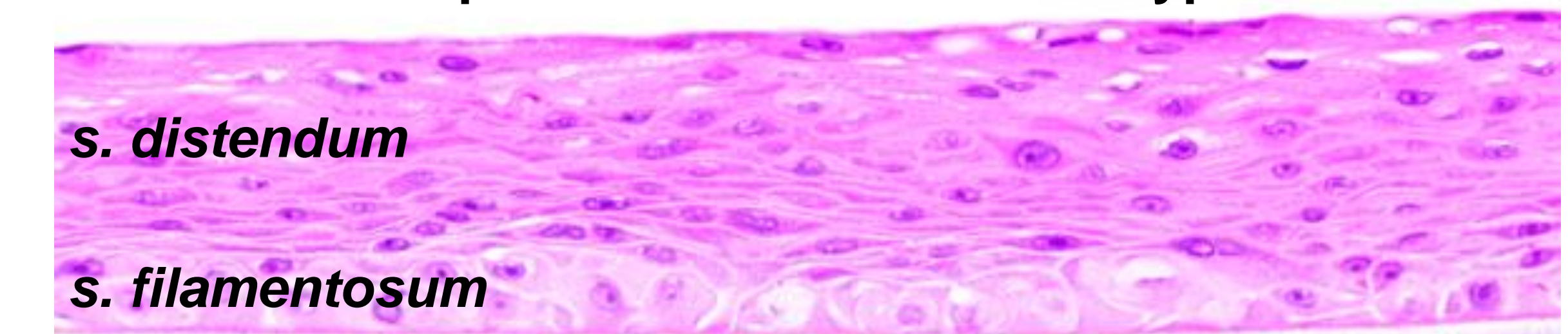
**Figure 2.** IL-1β cytokine data (ELISA) demonstrate the immune response of the tissues treated with each Magic Mouthwash after the 16 hour exposure. While all four mouthwashes induced higher concentrations of IL-1β compared to the 30 minute Negative Control (5.1 pg/mL), cytokine expression of MM 1 and MM 2 was significantly higher (44.9 pg/mL and 22.3 pg/mL respectively). No significant IL-1β activity was observed for the 5 minute and 30 minute exposures for any mouthwash.

Negative Control  
IL-1β = 5.1 pg/mL

Magic Mouthwash 1  
IL-1β = 44.9 pg/mL

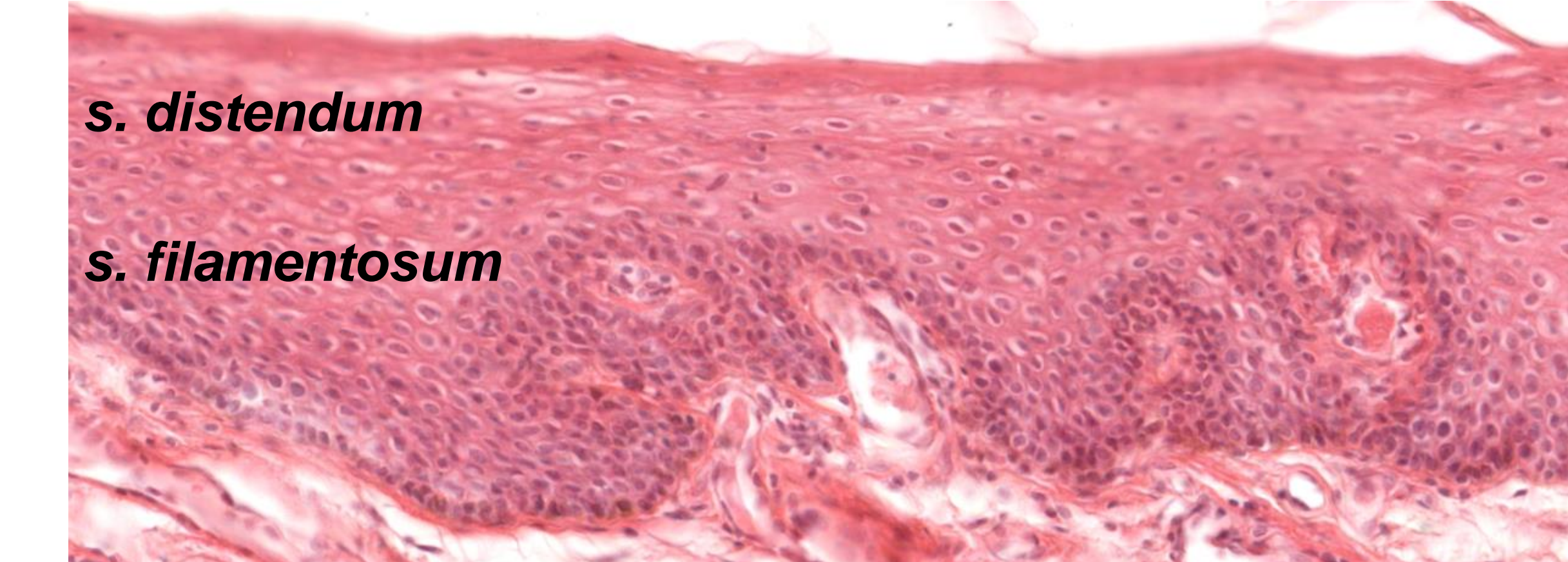
## Histology of 3D & Native Oral Tissue

### EpiOral™ 3D Buccal Phenotype



Histology is from MatTek Corporation of an EpiOral™ tissue model.

### Human Buccal Mucosa



Histology is from the Department of Cell & Developmental Biology, University of Michigan Medical School of the native human buccal mucosa.

While histology was not performed on the MM-treated tissues, these representative images of native and 3D buccal tissue provide visual representation of the similarities between these *in vivo* and *in vitro* models.

## Conclusions & Future Directions

### Magic Mouthwash 1 (MM 1):

- 33% Mylanta, AA used to coat the mouth and increase bioavailability.
- The only basic formulation tested (pH = 8).
- The most cytotoxic after 16 hours.
- Significant cell viability loss between short and long-term exposures (35% loss), and the highest inflammatory response (IL-1β = 44.9 pg/mL).
- The uniqueness and high concentration of Mylanta® indicates that the pH and cytotoxicity are likely driven by the antacid.

### Magic Mouthwash 2 (MM 2):

- The only Magic Mouthwash without lidocaine.
- No long-term toxicity was induced by MM 2.

### Magic Mouthwashes 2, 3, and 4 (MM 2, 3, 4):

- The anti-inflammatory steroids found in the composition of MM 2,3, and 4 correlated with reduced inflammatory response when compared to MM 1, which did not contain a steroid.

### Future Directions:

- The role of synergism requires further investigation, particularly surrounding the effects of lidocaine on the cytotoxicity induced to the tissues exposed to MM 1.
- The effects of the high concentration of antacid in MM 1 requires additional studies.
- The long exposure time needed to exhibit significant oral toxicity and inflammatory response supports the conclusion that the Magic Mouthwashes tested are safe when used as prescribed.
- Furthermore, the data promotes the use of non-animal methods based on reconstructed human tissue models to assess the safety of oral products for human use.
- Correlation with clinical endpoints is needed to gain confidence in the *in vitro* testing platform presented.