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IL-18 Sensitisation Assay in Labskin Tissues

OBJECTIVE

To determine Labskin's response as an *in vitro* skin model using an IL-18 / MTT based *in vitro* sensitisation assay comparing sensitising and non-sensitising materials.

METHODS

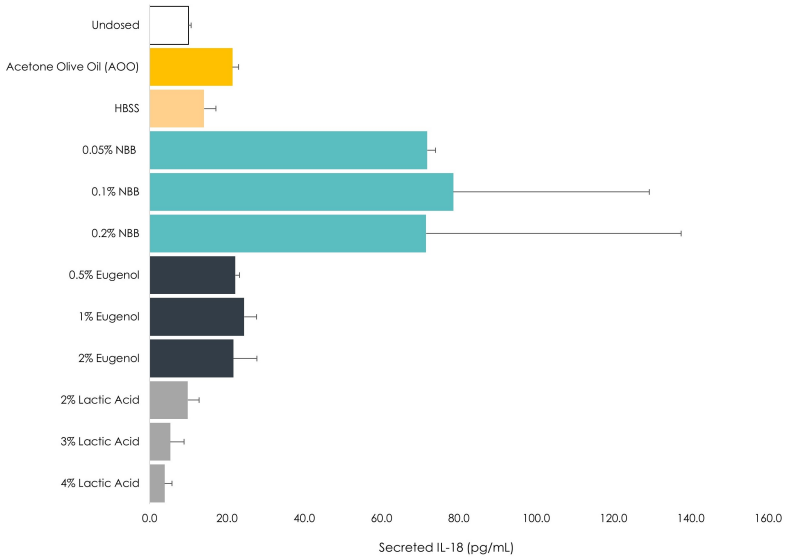
- Labskin Maintenance Medium (4.5 mL) was added to each well. The experiment was conducted using the 12 well plate configuration.
- Tissues were dosed (52 µL) with known sensitising materials (4-Nitrobenzyl bromide [NBB] and Eugenol) alongside a known non-sensitising material (Lactic Acid).
- Tissues were incubated with 1mL of MTT (1 mg/mL) in a 12 well plate for 3.5 hrs.
- Isopropanol (2 mL) was placed in each 12 well plate insert and then an additional 2 mL Isopropanol was added to the top of the tissues. Extraction occurred overnight.
- The following day 200 µL aliquots were removed and read by spectrophotometer (OD 540-690) for viability. The media was analysed by ELISA (MBL) for IL-18 secretion into the culture media.

RESULTS

The non-sensitiser Lactic Acid did not have any increase in IL-18 compared to vehicle control (AOO [Acetone: Olive OIL]), which is expected.

However, the sensitising material Eugenol did not have IL-18 increases over the vehicle control, which should have had a response. Fold increases in IL-18 for NBB was detected.

Figure 1 - IL-18 Secretion detected in culture media



SUMMARY

Overall, this experiment demonstrates that Labskin full thickness *in vitro* skin model is capable of responding to sensitising/allergic materials.

Labskin can be used within the same experimental design to evaluate several endpoints including cytokine responses (i.e. IL-1a, IL-6, IL-8, PGE2, TNFa, IL-10 etc.), histological changes, wound repair and photo-reactivity in addition to skin commensal and pathogenic microorganisms.

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labskin