

Chlorhexidine Cytotoxicity – Are There Safer Alternatives in Preoperative Skin Preparation?

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Introduction

- Chlorhexidine Gluconate (CHG)** is a broad-spectrum antimicrobial cleanser that is the standard of care pre-operative preparation solution to prevent surgical site infections¹, although current CDC guidelines consider the risk-to-benefit ratio an unresolved issue.²
- CHG is contraindicated for patients with insufficient epithelial barriers, including premature infants, infants < 2 months of age, and in certain skin diseases.
- Cases of severe desquamating adverse effects have been reported.^{3,4}

OBJECTIVE

Evaluate the relative cytotoxicity of a CHG-containing cleanser and two alternative skin cleansers, a baby shampoo and a colloidal silver-containing foam, in cultured pediatric epidermal keratinocytes and fibroblasts and measure the effects on proliferation, migration, and gene expression.

HYPOTHESIS

We hypothesized that CHG would be more cytotoxic to pediatric keratinocytes and fibroblasts *in vitro*, with greater inhibitory effects on proliferation and migration, and greater impacts on gene expression, compared with a baby wash and shampoo product and a colloidal silver-containing foam cleanser.

Methods

- Cells:**
 - Primary human keratinocytes and fibroblasts were cultured from 3 deidentified pediatric donors: aged 6 months (#905), 10 months (#980), and 12 years (#879).
 - Cells were used in experiments at passage 1 or passage 2.

- Cleansing agents:**
 - Cleansers analyzed were DynaHex4™ (CHG), Johnson's Baby Wash & Shampoo™ (BWS), and Theraworx Protect® with Colloidal Silver-containing Foam (CSF).

- Proliferation: MTT assay**
 - Keratinocytes and fibroblasts of each donor were cultured and treated with multiple dilutions of each agent. Dilutions of 1:1,000, 1:5,000, and 1:10,000 were analyzed in keratinocytes, and dilutions of 1:1,000, 1:2,500, and 1:5,000 were analyzed in fibroblasts. Concentrations were determined by preliminary experiments.
 - Each dilution was analyzed in quadruplicate for each cell strain.
 - Proliferation was measured at 24 hr and 48 hr via MTT assay.

- Cell Morphology: Digital photography**
 - Digital photographs were collected of cells treated for 24 hr with 1:5,000 dilutions of cleansing agents.

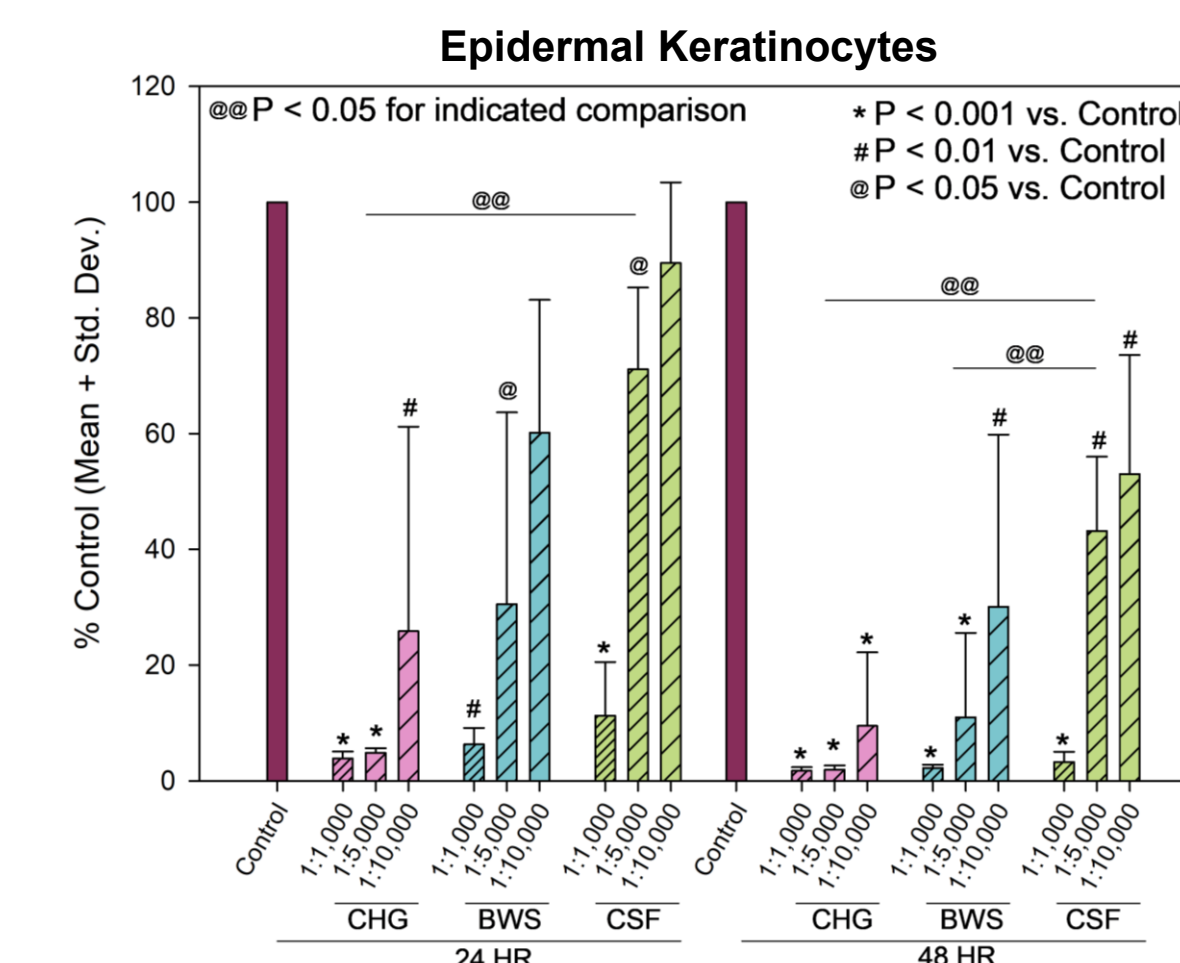
- Migration: *In vitro* "scratch" wound healing assay**
 - Keratinocytes and fibroblasts were cultured to confluence in duplicate prior to scratching each monolayer with a sterile 200 µl pipet tip to create a wound.
 - Culture medium containing 1:5,000 dilutions of cleansing agents was added, and photographs were collected at 0, 6, and 24 hr from three regions per scratch (N = 6 regions/condition/cell strain).
 - Image analysis (NIS Elements) was used to quantify the open areas at each time point and % wound closure was calculated.

- Marker Gene Expression: Real-time qPCR**
 - Keratinocytes or fibroblasts of each cell strain were treated for 24 hr with 1:5,000 dilutions of each cleansing agent.
 - Expression levels of marker genes were measured via real time quantitative PCR (qPCR):
 - Apoptosis:** C/EBP homologous protein (*CHOP*)
 - Inflammation:** interleukin 1B (*IL1B*)
 - Proliferation:** cyclin D1 (*CCND1*)
 - Fibroblast activation:** alpha smooth muscle actin (*ACTA2*) (fibroblasts only)

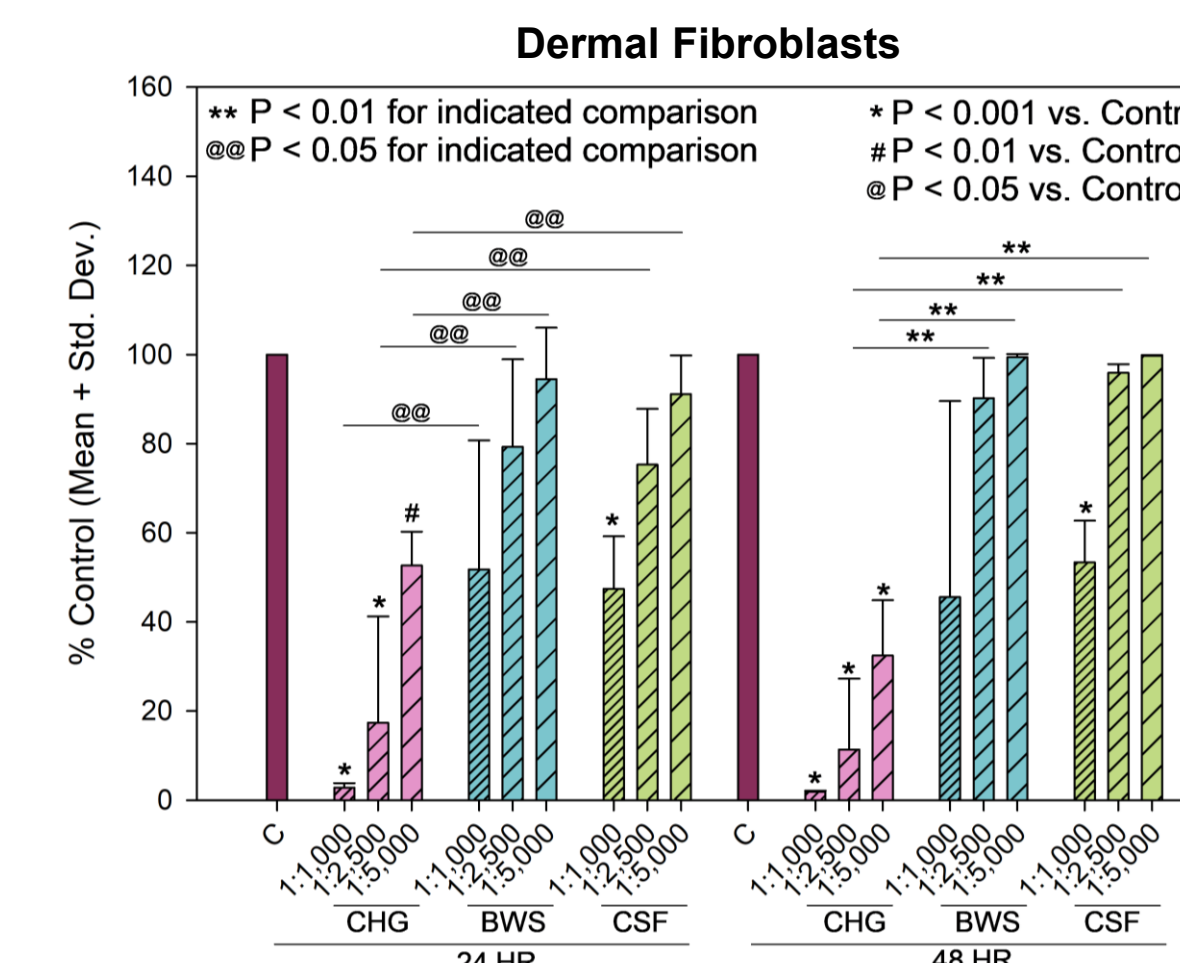
- Statistical Analysis: One-Way ANOVA**
 - Statistical analyses utilized One Way Analysis of Variance (ANOVA) and was performed using SigmaPlot 16.0.
 - Differences were considered statistically significant at P < 0.05.

Results

Proliferation:

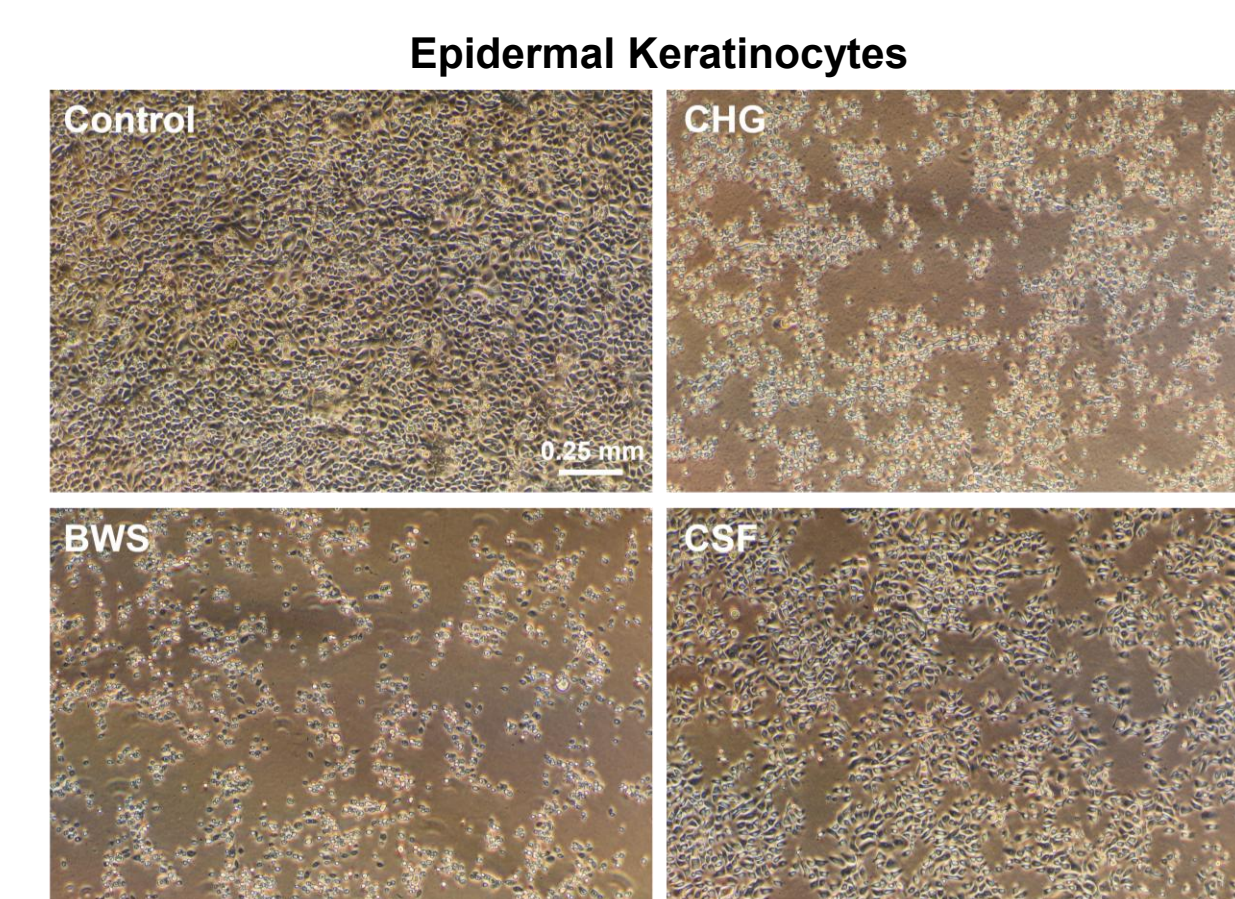


Keratinocyte proliferation: All agents significantly inhibited proliferation vs. controls at all dilutions at 48 hr and most dilutions at 24 hr. CSF had significantly less of an effect than CHG at 24 hr and both CHG and BWS at 48 hr.

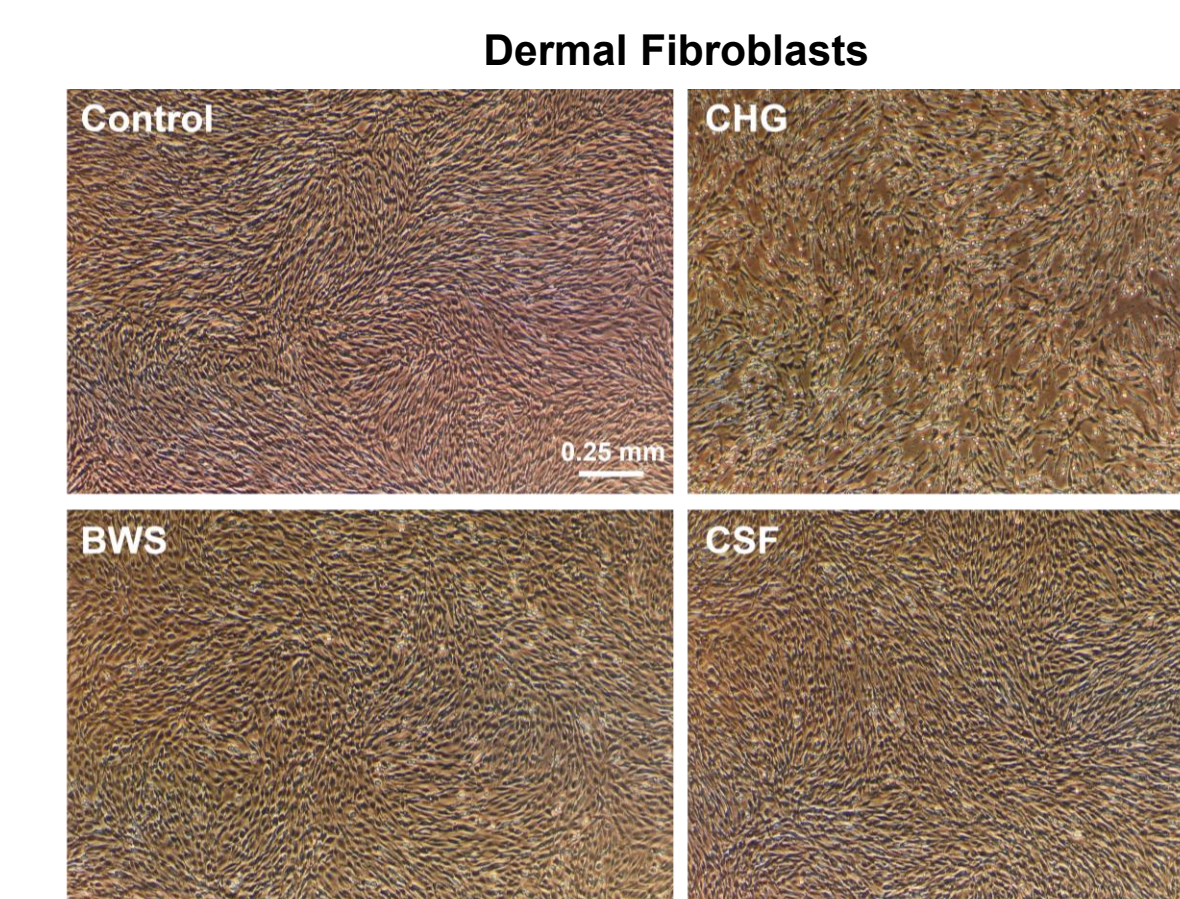


Fibroblast proliferation: CHG significantly inhibited proliferation vs. controls at all dilutions at 24 and 48 hr. BWS and CSF were significantly less toxic to fibroblasts than CHG when compared at similar dilutions.

Cell Morphology:

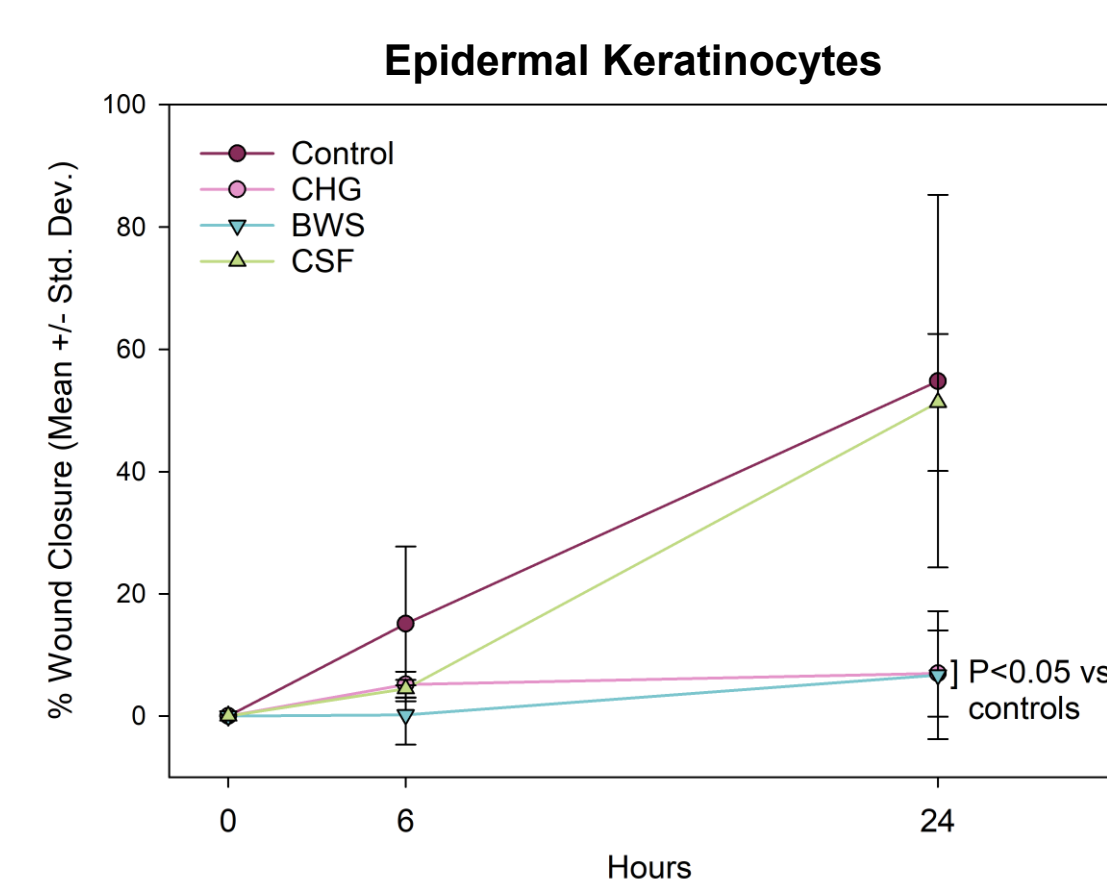


Keratinocyte morphology: Treated cells exhibited reduced numbers vs. controls. CHG and BWS-treated cells exhibited rounding and detachment of cells, indicating apoptosis or cell death; this was more pronounced with CHG treatment. CSF-treated cells exhibited a heterogeneous morphology compared with controls.

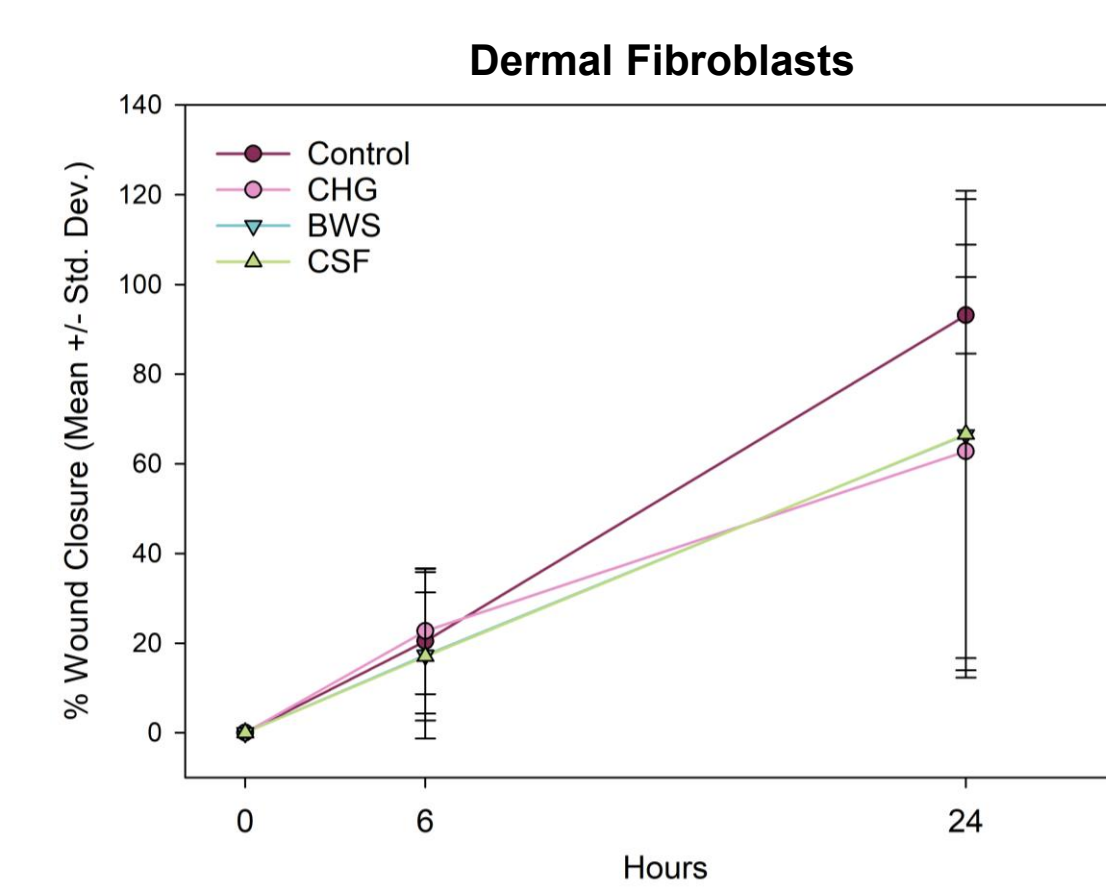


Fibroblast morphology: Cell numbers were decreased more dramatically vs. controls with CHG treatment vs. BWS or CSF. CHG caused blebbing, cell shrinkage, rounding, and detachment. Only minor blebbing and detachment were observed after BWS or CSF treatment.

Migration:

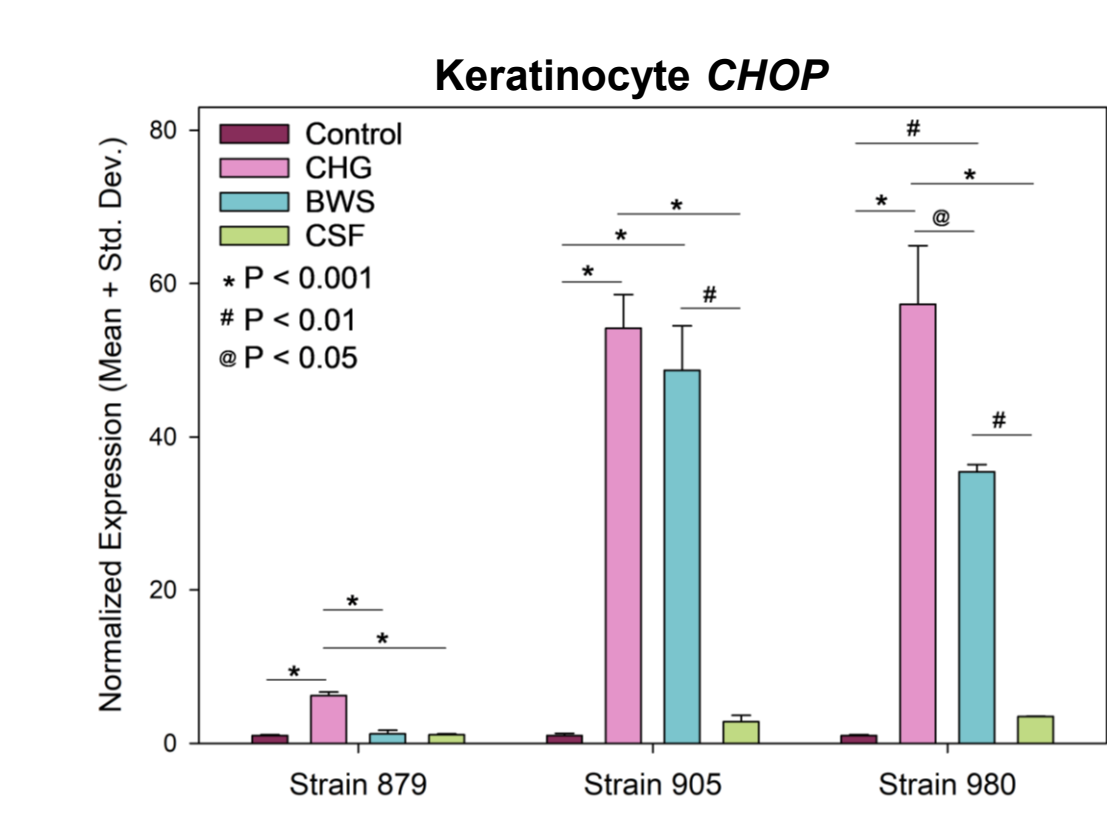


Keratinocyte migration: CHG and BWS significantly inhibited keratinocyte migration vs. controls. CSF had no significant effect on migration.

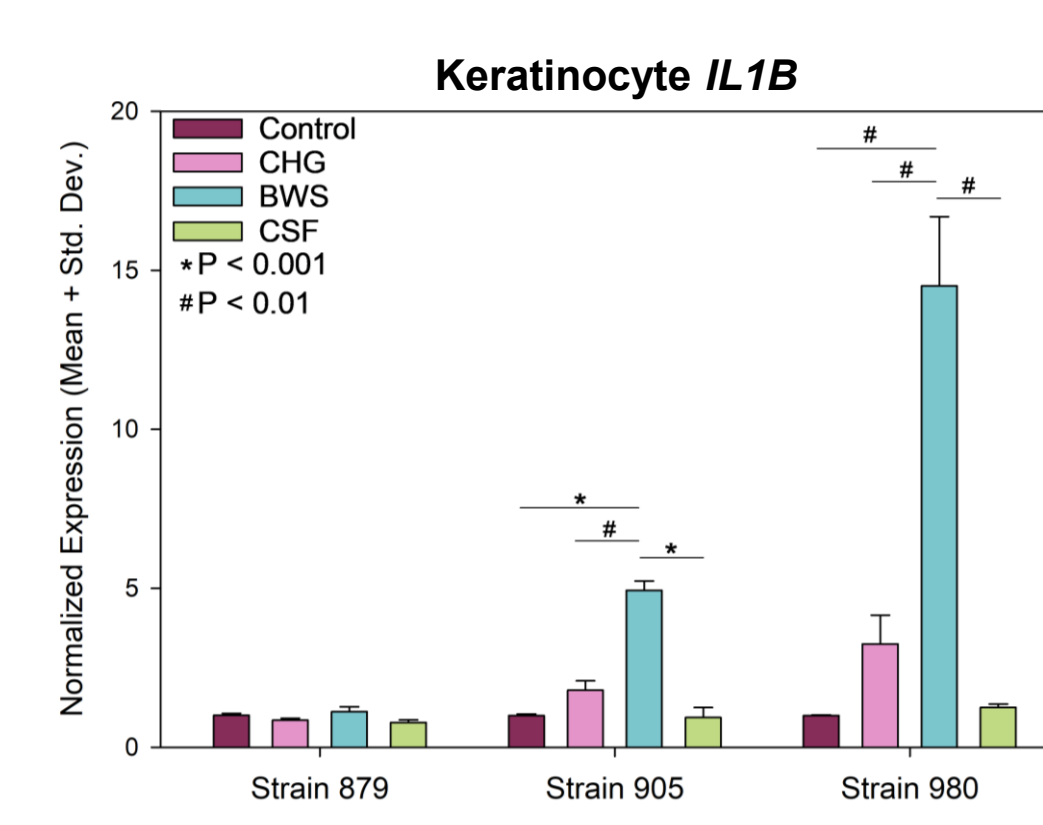


Fibroblast migration: A trend towards inhibition of migration was observed for all 3 cleansers; however, the results were not statistically significant due to variability among strains.

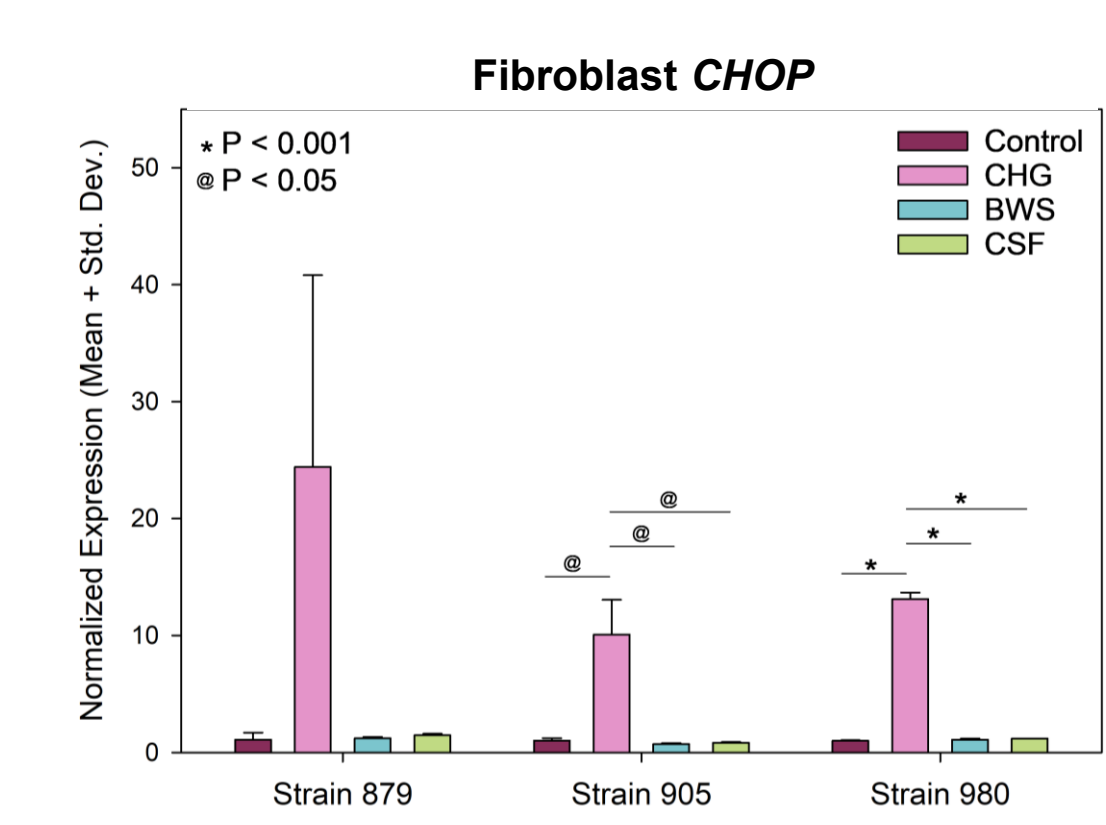
Gene Expression:



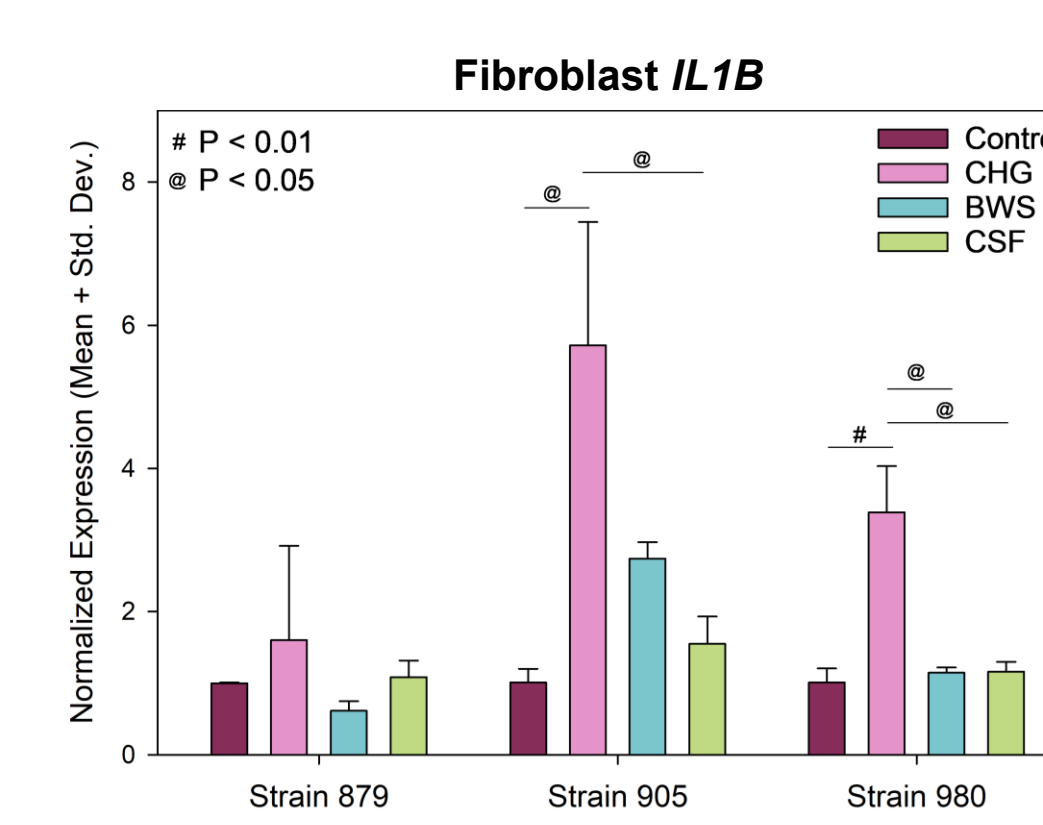
CHG significantly increased *CHOP* in all cell strains. BWS increased *CHOP* in cells of two donors. CSF did not increase *CHOP* in keratinocytes.



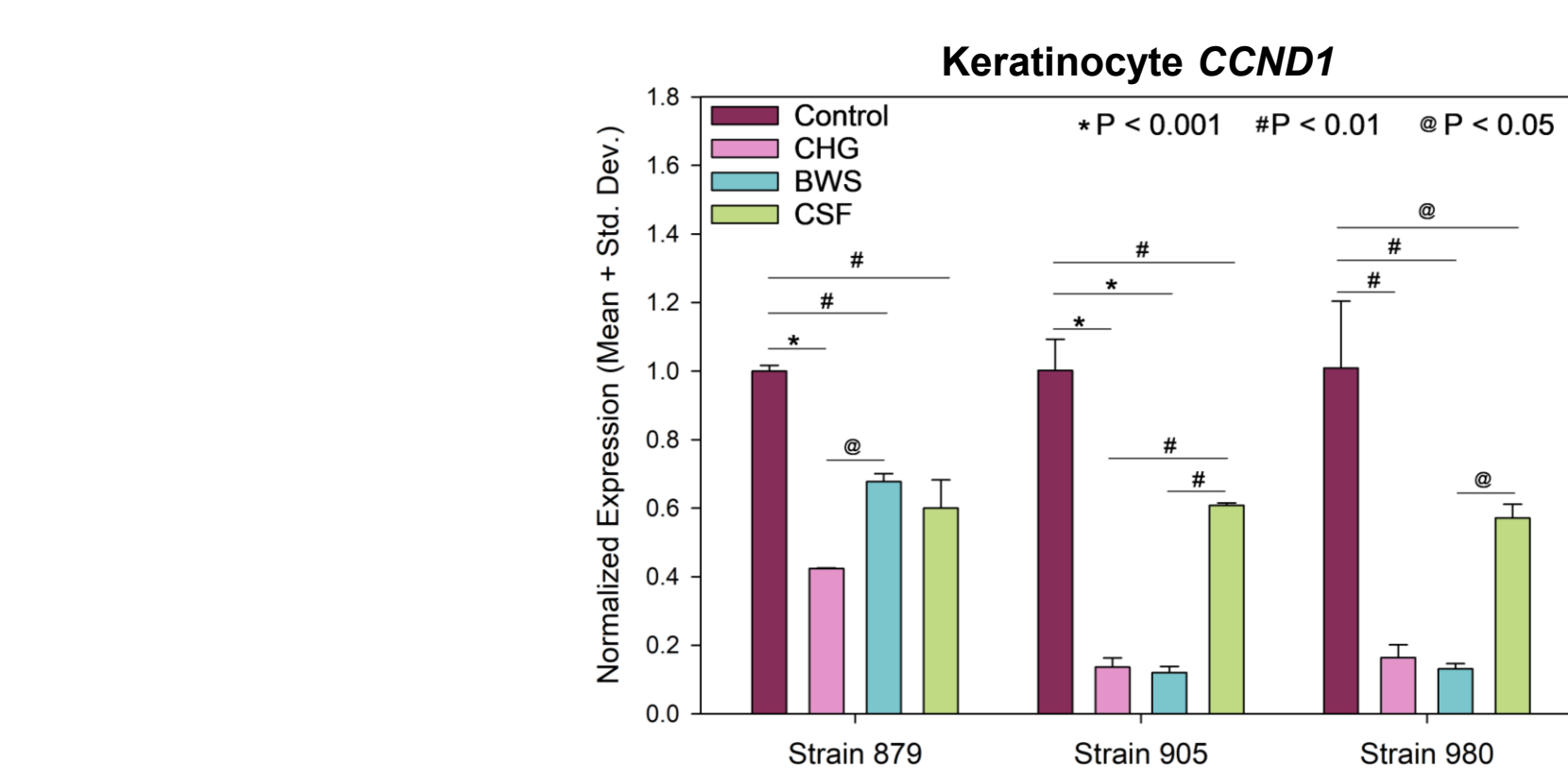
CHG and BWS increased *IL1B* in cells of two donors but this was only significant for BWS. CSF had no effect on *IL1B* in keratinocytes.



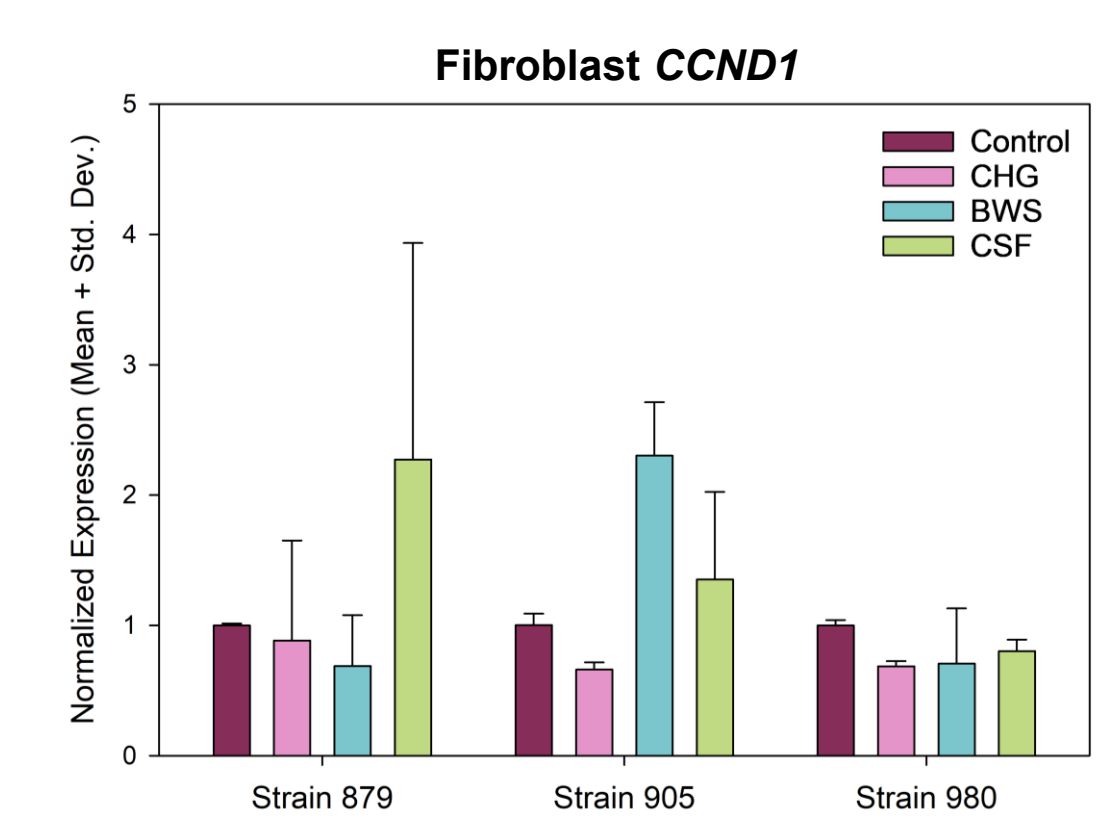
CHG increased *CHOP* but this was significant in cells of only two donors. BWS and CHG had no effect on *CHOP* expression in fibroblasts.



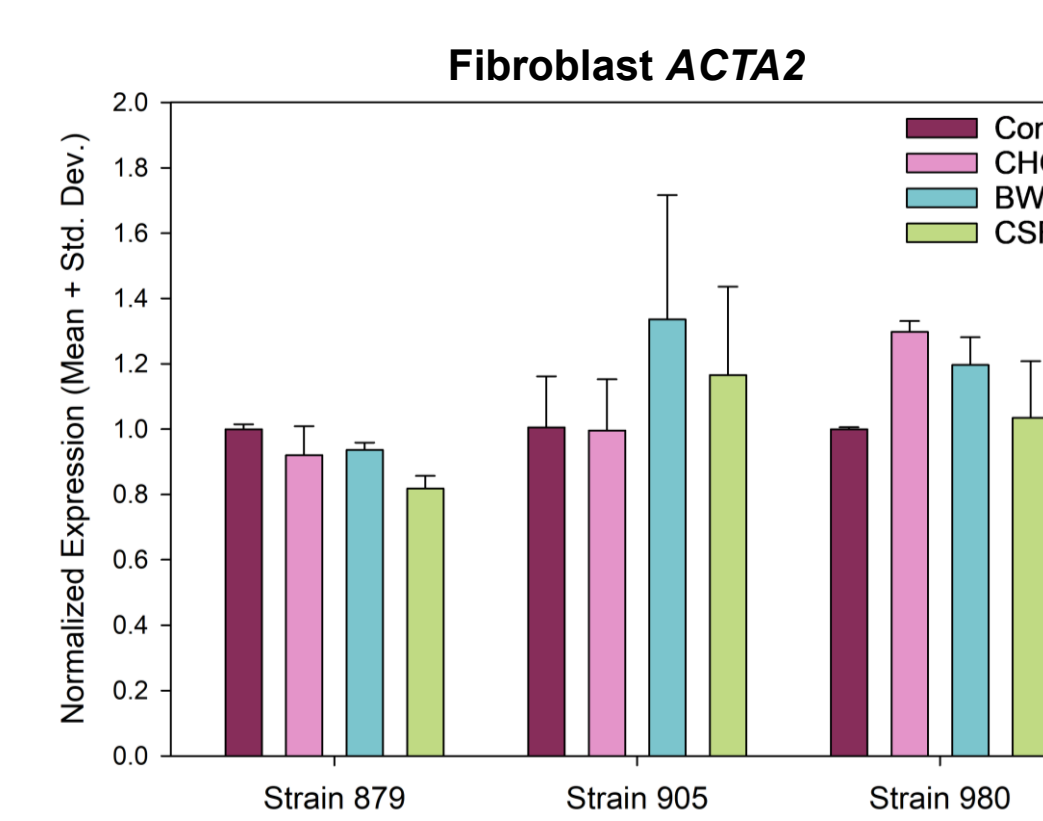
CHG significantly increased *IL1B* in cells of two of the three donors. BWS and CSF did not significantly increase *IL1B* expression in fibroblasts.



All three agents inhibited *CCND1* to varying degrees, with CHG and BWS having greater effects than CSF, and variability among strains.



None of the cleansers significantly affected expression of *CCND1* in fibroblasts.



None of the cleansers significantly affected expression of *ACTA2* in fibroblasts.

Conclusions

- Cleansing agents were variably toxic to primary pediatric keratinocytes and fibroblasts: CHG > BWS > CSF
- The results suggest the risk of at least some harm to skin cells when using CHG as a skin cleanser in the pediatric population.
- CSF caused the least damage to keratinocytes and fibroblasts, did not affect keratinocyte migration *in vitro*, and had the least impact on apoptosis, mitosis, and inflammation.
- Variability was observed between donor cell strains, which may be attributed to person-to-person differences in skin sensitivity.
- Surgeons and healthcare workers should be aware of the potential impacts of cleansers on skin cells and use caution when choosing an antiseptic cleanser, particularly in pediatric patients.

Limitations

- Although donor-to-donor variability was observed in response to cleanser exposure, the study was not sufficiently powered to detect differences due to any donor-specific factors (e.g., age, genetics).
- Only selected genes involved in inflammation, apoptosis, proliferation, and fibroblast activation were assessed. A broader analysis of gene expression will be required to fully characterize the responses of pediatric skin cells to these cleansers.
- Although *in vitro* assessments in cultured cells are standard and valuable, these results may not fully reflect responses of intact pediatric skin. *In vivo* studies would be more predictive of effects on whole tissues in patients.
- Microbiological assays were not performed. Analysis of relative antimicrobial activity will be required to fully define the optimal skin cleanser to effectively combat surgical site infections while causing the least damage to pediatric skin cells.

References & Acknowledgements

- References:**
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