STUDY OF MOISTURIZER CREAM CONTAINING A COMBINATION OF PREBIOTIC, PARAPROBIOTIC, AND POSTBIOTIC TOWARDS SKIN MICROBIOME

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ABSTRACT

Human skin is colonized by a diverse milieu of microorganisms, which could benefit the skin if they are balanced in amount and variety. The colony can be modulated by internal and external factors, including application of skin care products. Skin care containing microbiome ingredients can give efficacy to the skin, but also have potency to influence its microbiota ecosystem. The aim of this study is to evaluate the effect of cream containing a combination of prebiotic, paraprobiotic, and postbiotic towards *S. epidermidis*, *C. striatum*, and *C. acnes,* as 3 main microbiota in skin. The cream was evaluated on 3D skin cell model. Following the incubation, the undernatant was aspirated and frozen for quantification of IL-1α. Haematoxylin and Eosin (H&E) staining was used to assess skin cell morphology from all groups including untreated, dPBS control and treatment with the product. The cream showed no statistically significant effect on *S. epidermidis* and *C. striatum* compared to untreated control. On the other hand, *C. acnes* was significantly reduced. IL-1α released was not statistically significant compared to untreated or PBS control. H&E staining showed that the product gave no negative effect on skin model morphology when compared to untreated or dPBS controls. This study proved that moisturizer cream is considered as 'microbiome friendly' and unlikely to cause substantial irritation over 24-hour contact time with the skin model.

OBJECTIVE

To evaluate the effect of cream containing a combination of prebiotic (Saccharide isomerate), paraprobiotic (Bifida Ferment Lysate), and postbiotic (Hyaluronic Acid) towards *Staphylococcus epidermidis*, *Corynebacterium striatum*, and *Cutibacterium acnes.*

METHOD

1. Preparation of Moisturizer Cream Sample

1% Saccharide Isomerate, 1% Bifida Ferment Lysate, and 0.05% Hyaluronic acid was added into O/W cream emulsion base. The emulsion was mixed with Multimix homogenizer, resulting white opaque cream with pH around 4.5 - 5.5.

2. Preparation of 3D Skin Cell Model

Primary adult human dermal fibroblasts were embedded into a fibrin matrix to produce DEs. The DEs were cultured to allow the fibroblasts to remodel the matrix. The DE surface was attached by Primary neonatal human keratinocytes then cultured under liquid for 48 hours. The skin model was cultured at the air liquid interface (ALI) until a stratified epidermis was formed. Incubation conditions for all cultures was 37 ±2 °C in 5 ±1 % (v/v) CO 2 at ≥95% Relative Humidity (RH).

3. General Procedure and Assessment

Cutibacterium acnes NCTC 737, *Staphylococcus epidermidis* NCTC 11047 and *Corynebacterium striatum* NCTC 764 were cultivated on RCAF, MHA and ACA respectively at 37 ±2 °C. *C. acnes* was incubated anaerobic for 4 days while the rests were incubated aerobic for 24 hours.

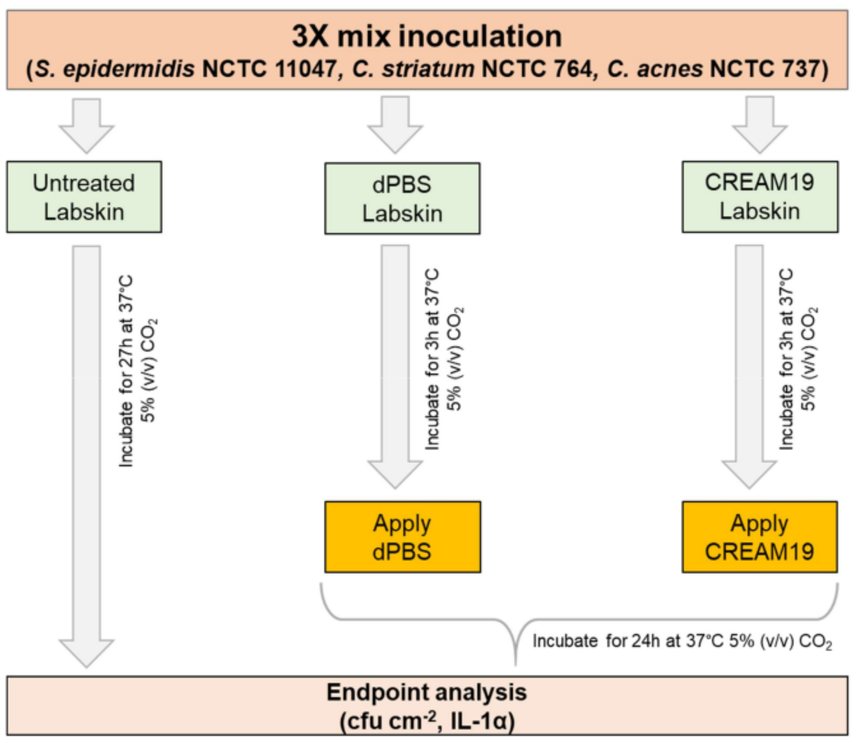


Figure 1. General Procedure and Assessment

Bacterial inoculum was prepared by mixing each bacterial colony with GS25 to create 1.1 x 10^6 CFU/ml. 10 µL of mixture bacteria (3X mix) was inoculated to colonize each skin model resulting in 10^4 CFU/cm² of each bacterium. All skin models were incubated at 37 ±2 °C in 5 ±1 % (v/v) CO 2 at ≥95% RH for 3 ± 1 hour. After initial incubation, the untreated group was left untreated; negative control (dPBS) was treated with 11 µL of dPBS; and the treated group (CREAM19) was treated with 11 µL CREAM19. incubated at 37 ±2 °C in 5 ±1 % (v/v) CO 2 at ≥95% RH for 24 ± 2 hours. Following the incubation, the undernatant was aspirated and frozen for IL-1α quantification. Biopsy samples of 8 mm in diameter were aseptically removed from the center of 5 (out of 6) skin models and viable microbial numbers were assayed on RCAF, MSA and ACA for *C. acnes, S. epidermidis* and *C. striatum* respectively.

4. Statistical Analysis

Statistical analysis performed in this study were Grubbs test, Shapiro-Wilks normality test, Ordinary one-way ANOVA with Tukey's multiple comparison test used for the post hoc analysis.

RESULTS

3D Skin Model Macroscopic Images

Macroscopic images of each 3D Skin Models were taken to see the consistency of procedure.

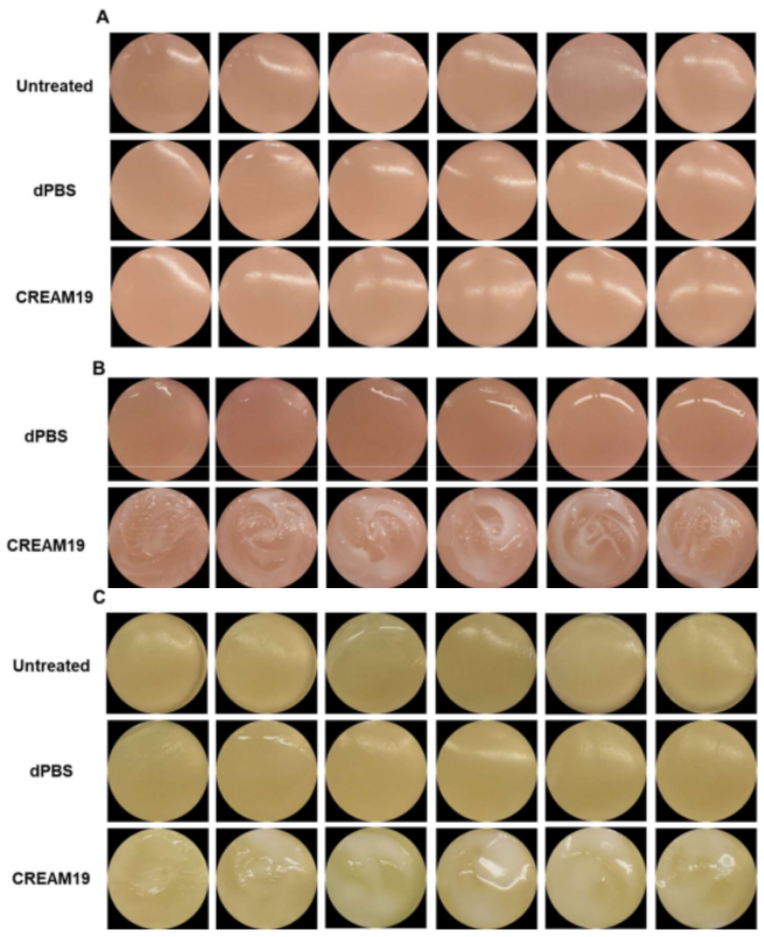


Figure 2. Macroscopic appearance of skin models. A. Pre-inoculation (T=0h), B. Post treatment with test items (T=3h), C. Post incubation (T=24h)

Bacterial Quantification

S. epidermidis showed no statistically differences between untreated groups and cream. *C. striatum* showed no statistically significant differences between any treatment groups. *C. acnes* showed no statistically significant differences between dPBS and cream. dPBS showed a statistically significant decrease of log ~1.01 in cfu cm -2 in comparison to the Untreated control (p=0.0169). The cream showed a statistically significant decrease of log ~0.95 in CFU/cm² in comparison to the Untreated control (p=0.0153).

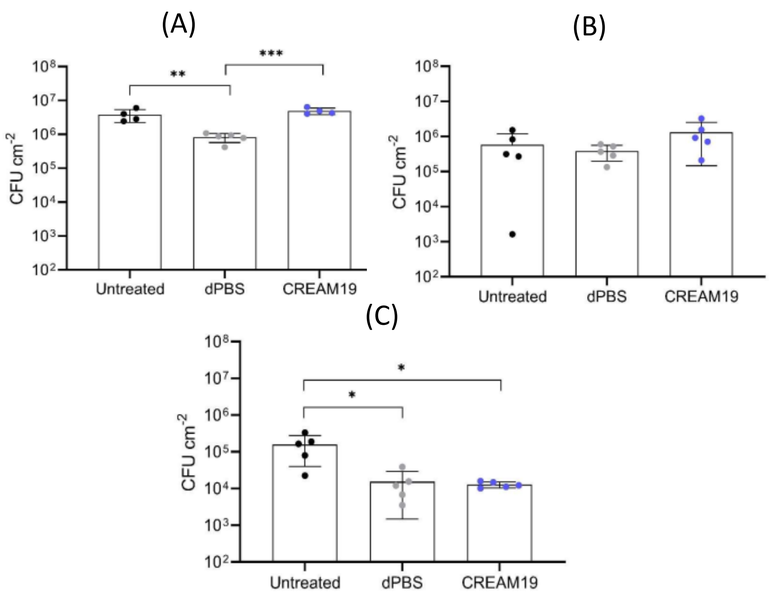


Figure 3. Cfu/cm² of bacteria recovered from skin models. (A) *S. epidermidis,* (B) *C. striatum,* (C) *C. acnes*

Immunology

Immunological responses were assessed by the quantification of inflammatory cytokine release into the Skin Model maintenance media. This release construes part of the skin's response to stimuli on the surface or within the tissue. IL-1α released from the dPBS control is the result of application and spreading of the dPBS control which causes slight mechanical damage. The cream did not cause a statistically significant inflammation when compared to Untreated or dPBS control.

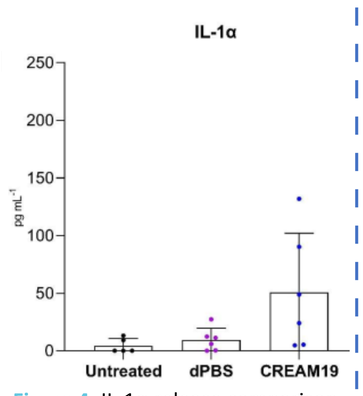


Figure 4. IL-1α release comparison

Histology

Haematoxylin and Eosin (H&E) staining was used to assess Skin model morphology. Different levels of bacterial colonization could be observed on the stratum corneum of all replicates tested. The cream had no visible effect or damage on the cutaneous structure of Skin Model when compared to Untreated or dPBS controls.

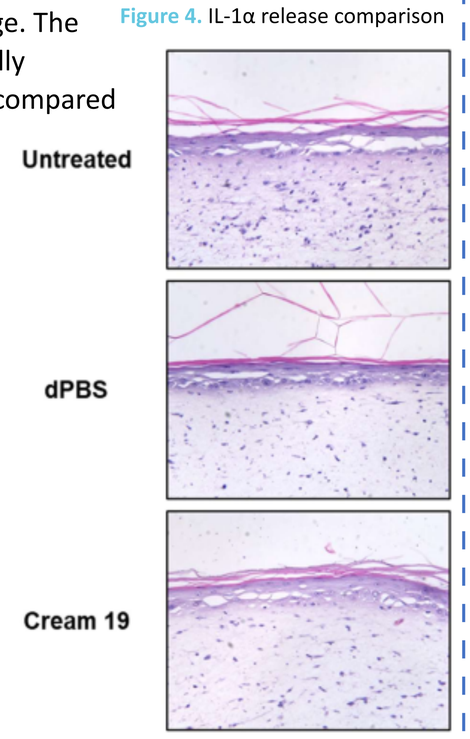


Figure 5. Skin model morphology

CONCLUSION

Prebiotic (Saccharide Isomerate), paraprobiotic (Bifida Ferment Lysate), and postbiotic (Hyaluronic Acid) were added to the cream formulation to have several benefits, including moisturizing and DNA repair stimulation. In addition, this product is expected to have microbiome friendly properties. 'Microbiome Friendly' means that the product does not disturb the microbial balance of the skin and helps preserve the diversity of the natural microbiome. This study proved that moisturizer cream is considered as 'microbiome friendly' as shown by no statistically significant effect on *Staphylococcus epidermidis* and *Corynebacterium striatum*, and the microbiome diversity is still maintained although there is a decrease in *Cutibacterium acnes* number. The product is unlikely to cause substantial irritation over 24-hour contact time with the skin model.