Revolutionizing Modern Skin Care with an Old Technology: Phage Therapy in the Age of the Skin Microbiome

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ABSTRACT.

Background: As the skin care industry pursues technologies that improve skin health through modulation of the skin microbiome, it will be forced to contemplate the growing tide of ineffectual anti-microbials. Scientists across multiple disciplines have focused on developing antibiotic alternatives with the leading candidate being "phage therapy". With an understanding of how critical a balanced skin microbiome is to skin health; phage therapy represents a safe and powerful tool to exert changes in microbial constituents.

Methods: The capacity of three bacteriophages to diminish *C. acnes* was evaluated in bacterial cultures and a reconstituted human epidermal model infused with *C. acnes* to replicate blemish-prone skin. Potential cytotoxicity of the phages was also tested on keratinocytes and fibroblasts.

Results: A volume of phage less than one-tenth of the bacterial culture reduced associated turbidity by greater than 75% in less than 24 hours. Similarly, in the acne-prone skin model, the phage down-modulated the bacteria and reduced inflammation with no ill effects to skin cells. The phage combination was examined for compatibility in various formulations, where activity was diminished in formulations containing high amounts of 1,3-propanediol. In contrast, water-based solutions containing phenoxyethanol or 1,2-alkanediol/hydroxyacetophenone had no negative impact on the phages to eliminate their target bacteria.

Conclusion: Phage therapy represents a new methodology for modulating the skin microbiome. This platform is effective at reducing targeted bacteria without collateral damage to beneficial neighboring species. Topical application of phage-infused formulations delivers a one-two punch of countering *C. acnes* and repairing damage from associated inflammation.

Keywords:

bacteriophage; skin microbiome; acne vulgaris; *Cutibacterium acnes*

INTRODUCTION.

In recent years, there has been a remarkable and understandable surge of interest in the human microbiome – the full complement of commensal microbes that have colonized multiple parts of the human body and have significant impacts on our health. Research has elucidated profound roles for a multitude of microbial constituents in a series of tissues, including but not limited to the gastrointestinal tract, lungs, blood, brain, tumors, and skin. In the context of the skin, these microorganisms comprise species from all three domains of cellular life: Eukarya, Bacteria, and Archaea. Modern amplicon sequencing techniques have greatly facilitated the identification of these resident microbes and their relative biogeography among the various regions of human skin. Furthermore, metagenomic sequencing that does not rely upon established host genes, has expanded this list to include an entire series of viruses, the largest member population being the bacteriophages.

Bacteriophages are viruses that target bacterial cells in a species-specific manner (1). Unlike their eukaryotic counterparts, they possess highly complex capsid shells that have a tendency to resemble the construction of early lunar lander probes. A further distinction of these bacterial viruses is that their genetic material tends to be composed of deoxyribonucleic acid (DNA) rather than ribonucleic acid (RNA). This preference is switched for eukaryotic viruses, where RNA-based genomes dominate over DNA-based ones. Upon attachment to a cognate host bacterium, a needle-like component of the bacteriophage capsid will puncture through the peptidoglycan cell wall of the bacterium, and the genomic DNA will travel down

a channel from the capsid head through the needle appendage into the bacterial cytoplasm. At this point, the bacteriophage life cycle can progress down one of two separate paths: lytic phase or lysogeny. With lysogeny, a pause occurs upon delivery of the bacteriophage genome into the cytoplasm. During this pause, single or multiple copies of the bacteriophage genome are produced called "prophages" that are subsequently integrated into the host genomic DNA. The danger posed by this path is that the prophages can transduce the bacterium with new genetic material that might evolve the pathogenicity of that microbe (1). A detrimental example being the introduction of an antibiotic resistance gene or a toxin gene. At a later point, the dormant bacteriophage genomes can become reactivated and the bacteriophage life cycle will enter the lytic phase. Bypassing lysogeny and committing to the lytic phase is preferred for anti-bacterial applications. In this case, translation and replication of the genomic DNA immediately commence, culminating in the assembly of multiple replicate bacteriophage particles that will eventually lyse and rupture the bacterial cell. On average, roughly 1000 bacteriophage particles are generated from the infection of a single bacterium by a single virus. This exponential growth pattern is the reason for why viruses will always outnumber cellular microbes in the human microbiome as well as in the surrounding environment.

The effectiveness of lytic bacteriophages at combating bacterial infections, so-called "phage therapy", was first described by the studies of two World War I microbiologists: Doctors (Drs.) Frederic Twort and Felix d'Herelle, more than thirty years prior to the advent of antibiotics (2, 3). When antibiotics were discovered and developed in the 1940s, the Western world largely abandoned phage therapy with only the former Soviet Union maintaining active phage research (4). Contemporarily there is renewed interest in this more than century old technology as many bacterial pathogens that were once kept under control by antimicrobial compounds have evolved resistance to these previously effective countermeasures (5, 6). These acellular biological entities represent the most likely candidate intervention to counter the rising scourge of antibiotic resistant bacteria (1, 6, 7).

The first widespread application of phage therapy in the modern age was in the context of anti-microbial food sprays to prevent further outbreaks of bacteria-caused food-borne

illnesses such as *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli*. (8-13). Since this approach means that the consumer will eventually be eating foods coated with bacteriophages, regulatory agencies have tacitly bestowed these bacterial viruses with the moniker of "generally recognized as safe" or "GRAS". From these successes, many academic and industrial groups have started devising safe, high-throughput manufacturing processes and enhanced delivery mechanisms for phage therapy (14-16). There has been an explosion of interest beyond pharmaceutical applications to the cosmetic care industry and in some cases, probiotic uses.

In the era of the Human Microbiome Project, bacteriophages have also become an attractive means by which to carefully modulate the characterized human microbiota. Indeed, there are many benefits to the utilization of bacteriophages as a mechanism of exerting changes in the microbial constituents of the skin microbiome. For one, unlike antimicrobial compounds, bacteriophages are exquisitely species-specific and in some cases strain-specific. That targeted nature allows the microbial culprits of various skin conditions to be diminished without any collateral damage to the healthy commensals also present. Second, very little bacteriophage is required to be administered to have a profound impact given their capacity for exponential replication – as stated above, on average, a single phage particle infecting a bacterial cell will result in approximately 1000 progeny viruses. Third, the bacteriophage infection is self-limiting in that once there are no longer any susceptible bacterial present, the viruses will cease to propagate and self-eliminate. Finally, they have an excellent safety profile given that millions of years of evolution separate bacteriophages from the viruses of eukaryotic cells, rendering them incapable of infecting human skin cells.

Here, a combination of three novel bacteriophages targeted to *Cutibacterium acnes* was investigated for its capacity to down-modulate levels of this bacteria on the skin and by extension to ameliorate acne vulgaris, the most common skin affliction worldwide. The *C. acnes* bacteriophages were tested for their efficacy and safety in bacterial cultures, monolayers of human skin cells, in acne-prone three-dimensional skin model systems, and finally in a pilot clinical study. The outcomes of these investigations will expand upon the knowledge from previous efforts to adapt phage therapy for anti-acne applications (17-19).

MATERIALS AND METHODS.

Materials. Peptone yeast extract broth with glucose (PYG), cooked meat medium (CMM), and Columbia blood agar plates were purchased from Anaerobe Systems (Morgan Hill, CA, USA). Bacteriophages targeted to *Cutibacterium acnes* were isolated and purified by JAFRAL (Ljubljana, Slovenia). Plaque assay kits were purchased from Edvotek (Washington, DC). Gas packs to create anaerobic culture conditions were purchased from Becton-Dickinson Diagnostics (Franklin Lakes, NJ, USA). Resazurin reagent was purchased from Sigma-Aldrich (Saint Louis, MO, USA).

Bacterial culture. *Cutibacterium acnes* (strain 6919) was procured from American Type Culture Collection (ATCC; Manassas, VA, USA). The bacteria were initially cultured in CMM broth per ATCC specifications at 37˚ C with shaking under anaerobic conditions for no less than 2 days. Subsequently, to avoid animal-derived materials, the bacteria were successfully cultured to higher levels in PYG broth anaerobically at 37˚ C with shaking for 2 days. The degree of growth was assessed by spectrophotometry (see below). Colony morphologies were evaluated by streak plating on Columbia blood agar and incubating for 2 days at 37˚ C under anaerobic conditions.

Mammalian cell culture. The keratinocyte-derived HaCaT cell line and normal human dermal fibroblasts (NHDF) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and were cultured in Dulbecco's Minimal Essential Media (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% HEPES, and 1% penicillin/streptomycin at 37 ˚C in 5% CO_2. Normal human keratinocytes (nHEK) were procured from Thermo Fisher Scientfic and were cultured in EpiLife™ media with human keratinocyte growth supplement (HKGS) at 37 ˚C in 5% CO_2.

Bacteriophage hunt. The search for *C. acnes* specific bacteriophages was outsourced to and conducted by the JAFRAL group (Ljubljana, Slovenia).

Spectrophotometry assessment. Initial evaluations of the efficacy of the individual bacteriophages (and in combination) to counter the growth of *C. acnes* were conducted using a standard spectrophotometer observing the optical density of various preparations at 600 nanometers (nm) – OD_{600}. Briefly, a culture of *C. acnes* would be grown to confluence over

the course of 2 days, at which time less than one-tenth volume of bacteriophage preparations would be inoculated. The OD_{600} of the culture would then be measured every 3 hours (h) for no longer than 24 h total.

Cytotoxicity assay. Multiple preparations of the three bacteriophages targeted to *C. acnes* were applied to monolayers of multiple skin cell cultures including NHDF, nHEK, and HaCaT cells. After incubating on the different human skin cell cultures for a period of 72 h, the cells were visually examined for any morphological changes commonly associated with cellular stress. The exposed cells were also tested by a resazurin assay to quantify any cellular cytotoxicity in response to incubation with the three bacteriophages. Briefly, all cell treatments were aspirated and cells were washed twice with Hank's Balanced Salt Solution (HBSS). The final wash HBSS was aspirated, replaced with 42 µM resazurin salt solution and allowed to incubate at 37˚ C with 5% CO_2, until a color change was observed. Upon the detection of a color change, it was compared to the starting solution color, where 200 µL of each sample was placed into a clear, flat bottom 96-well plate and the absorbance was read at both 570 nm and 690 nm.

Plaque assay. Purified bacteriophages specific for *C. acnes* were mixed into a variety of formulations and preparations. In parallel, cultures of *C. acnes* were grown to confluence. When ready, the phages were mixed with the bacteria cultures, and a plaque assay was conducted per manufacturer's (Edvotek) instructions. Luria broth (LB) base plates were prepared upon which a molten (heated to ~65 ˚C), red-dyed soft-agar overlay containing 1 mL of *C. acnes* (OD_{600} ≥1.0) and 100 µL of test bacteriophage material was poured and allowed to solidify at room temperature for approximately 20 min. Phosphate-buffered saline (PBS) was substituted for bacteriophage as a negative control, and all bacteriophage dilutions were prepared using PBS. The plates were then placed in an anaerobic chamber with an anaerobic gas pack and allowed to incubate at 37 ˚C for 24 h. The following day, all plates were inspected for plaques, which were enumerated and the relative plaque-forming units per milliliter (PFU/mL) were calculated for each of the test plates.

Reconstituted human epidermal (RHE) blemish-prone skin model. The triple phage combination targeted to *C. acnes* was submitted to StratiCELL (Les Isnes, Belgium) for testing on their RHE acne model system. Briefly, the RHE was constructed with an infusion of *C. acnes* phylotype IA1. The growth levels of *C. acnes* on the RHE stratum corneum were

determined by quantifying the number of colonies on media plates inoculated from the RHE. Five concentrations of the *C. acnes* bacteriophage combination were applied to the skin model to histologically confirm a non-cytotoxic dose range. The impact on the infused bacteria was assessed by standard plaque assay and a series of inflammatory factors were evaluated for changes in expression levels in response to the bacteriophage combination by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA).

Electron microscopy. Transmission electron microscopy (TEM) was utilized to evaluate the shape and size of the three (3) different bacteriophages targeted to *C. acnes*. In a collaboration with the ThiNC (Thermomechanical and Imaging Nanoscale Characterization) group at Stony Brook University (Stony Brook, NY, USA).

Pilot Clinical Study. A formulation infused with the triple *C. acnes* bacteriophage combination in water with 0.7% phenoxyethanol was supplied to a clinical studies organization (Validated Claims Support, Teaneck, NJ, USA) to evaluate on six (6) participants for its capacity to reduce *C. acnes* associated coproporphyrin III fluorescence and skin redness/blemish. High-resolution images were collected of the participants at multiple camera angles at the start of the study and 7 days after applying the triple *C. acnes* bacteriophage combination to the face twice daily.

RESULTS.

Individually and in combination, three bacteriophages targeted to C. acnes significantly diminish the bacterial population. At the inception of the investigation into the utility of so-called "phage therapy" to modulate the population of a specific microbial constituent of the skin microbiome, a search for bacteriophages uniquely targeted to *C. acnes* was conducted in Slovenia (courtesy of the JAFRAL group). A minimum of three (3) bacteriophages was desired to reduce the possibility of the target bacteria adapting through coordinated mutation to the viral treatment (16). Three unique bacteriophages (phages) were collected, which were then examined for their capacity to function as lytic phages of *C. acnes* by both optical density measurements at 600 nm (OD_{600}) and standard plaque assays.

The individual phage preparations (#1, #2, and #3) were separately evaluated for their capacity to reduce the OD_{600} of a turbid culture of *C. acnes* – culture OD_{600} prior to phage introduction was approximately 0.75-0.90. As shown in Figure 1A, increasing volumes of each phage dose-dependently reduced the OD_{600} of the *C. acnes* culture. Similarly, the three phages in combination were tested for their ability to reduce the OD_{600} of the *C. acnes* culture. The triple phage treatment (100 µL into a 50 mL *C. acnes* culture) resulted in a greater than 60% reduction in the OD_{600} value (Figure 1B). It is important to consider that uninoculated media often resulted in an OD600 of as little as 0.10-0.15. The reductions observed for the OD_{600} were corroborated by standard plaque assay, which was also used to determine the relative titers of each phage preparation (data not shown). Based on these preliminary findings, it was concluded that the three different phages discovered were effective at diminishing *C. acnes* populations both individually and in combination.

FIGURE 1

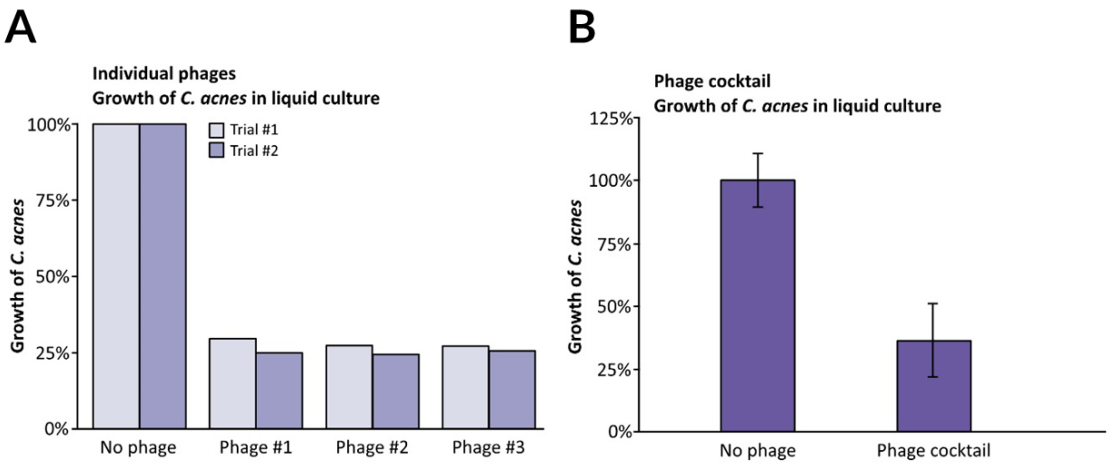

Figure 1. Three unique bacteriophages targeted to *C. acnes* significantly diminish the bacteria species. When evaluated by OD_{600}, reduced *C. acnes* levels were quantified upon 24 h incubation with all three bacteriophage candidates individually (A) and in combination (B). Note that *C. acnes* cultured alone produced an OD_{600} range of 0.75-1.5, while uninoculated media produced an OD_{600} range of 0.10-0.15.

Triple C. acnes bacteriophage preparation is non-cytotoxic under multiple test conditions. Millions of years of evolution separate the viruses that infect eukaryotic cells from the bacteriophages that infect prokaryotic cells or bacteria, effectively rendering them incapable of infecting cells that are not part of their unique domain of cellular life. Despite that, the

bacteriophage preparations shown to target *C. acnes* were evaluated for their ability to cause any form of cytotoxic effect when applied to monolayers of human skin cells. To that end, different preparations of the triple combination of bacteriophages described previously were applied to cultures of normal human keratinocytes (nHEK), a keratinocyte-derived cell line (HaCaT), and normal human dermal fibroblasts (NHDF) over a period of 72 h. Visual inspection post-incubation revealed no morphological alterations to the cells that would suggest toxicity or stress such as cell rounding or detachment. Afterwards, the cells were tested for any indications of cytotoxicity via a resazurin assay (described in Materials and Methods section). At all of the concentrations tested, no cytotoxic effect was detected for any of the *C. acnes* phage preparations (Figure 2).

FIGURE 2

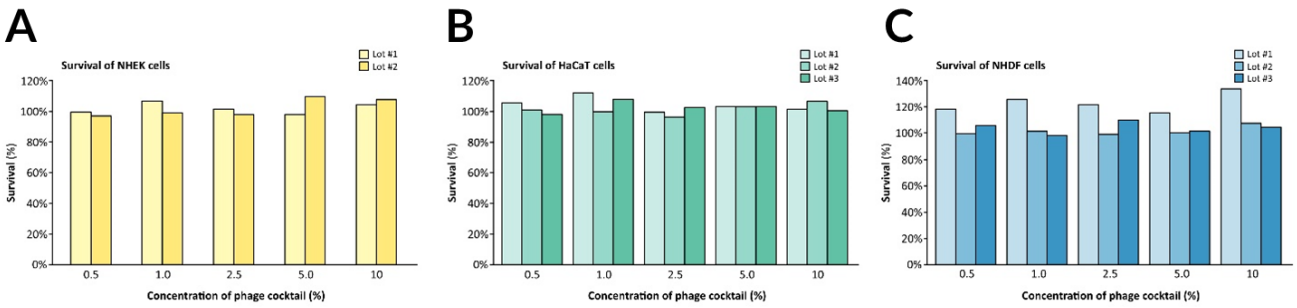

Figure 2. The combination of *C. acnes* specific bacteriophages was shown to be non-cytotoxic when applied to monolayers of multiple human skin cell cultures and incubated for 72 h: nHEK (**A**), HaCaT (**B**), and NHDF (**C**) as quantified by a resazurin cytotoxicity assay.

The triple* C. acnes *phage combination is effective and safe when applied to a three-dimensional model of acne-prone human skin. A reconstituted human epidermis (RHE) model of acne prone human skin was used to examine both the safety and efficacy of the triple phage solution that had been demonstrated to rapidly lyse *C. acnes* populations and be non-cytotoxic to human skin cells *in vitro* (Figures 1 and 2). The effort was divided into three (3) evaluations: 1) safe dose determination, 2) impact on *C. acnes* growth, and 3) impact on skin inflammation.

For the first task, five dilutions of the triple phage combination were applied to the *C. acnes*-infused RHE and were examined histologically with hemalum/eosin staining for any indications of toxicity to the tissue, relative to a stressful 0.1% SDS positive control for cytotoxicity. As shown in Figure 3A, no gross histological changes were visualized in the phage treated samples relative to untreated controls at any of the five doses tested. Only with the 0.1% SDS treatment was a morphological aberration observed with thickening and overall disorganization of the stratum corneum layer. This supported the conclusion that the phage combination was in no way noxious to the tissue.

The next evaluation to determine if the phage mixture had an impact on *C. acnes* growth was conducted after the phage had been incubated topically on the RHE for 72 h. Any remaining bacteria was collected by swabs and then streaked onto enriched media plates that were incubated for 5 days anaerobically. The phage treatment affected a notable decrease in *C. acnes* colonies as illustrated in Figure 3B, corroborating the lytic potential of the phage combination that was previously seen in laboratory bacterial cultures.

Lastly, the RHE tissue was examined for any alterations in the levels of four (4) acne-associated inflammatory factors: interleukin-8 (IL-8 or CXCL8), macrophage inflammatory protein-1 beta (MIP-1β), monocyte chemoattractant protein-1 (MCP-1), and interleukin-1 beta (IL-1β). IL-8, MIP-1β, and MCP-1 levels were quantified by RT-qPCR, and IL-1β was tested by ELISA. These tests revealed that there was a significant decrease in IL-8 levels in response to the phage combination treatment, relative to the no phage control that exhibited spiked IL-8 concentration in response to *C. acnes* growth (Figure 3C). MIP-1β expression was not detected in any of the samples tested including the controls. Minor reductions in MCP-1 and IL-1β were observed, but were low enough to question their substantiation.

FIGURE 3

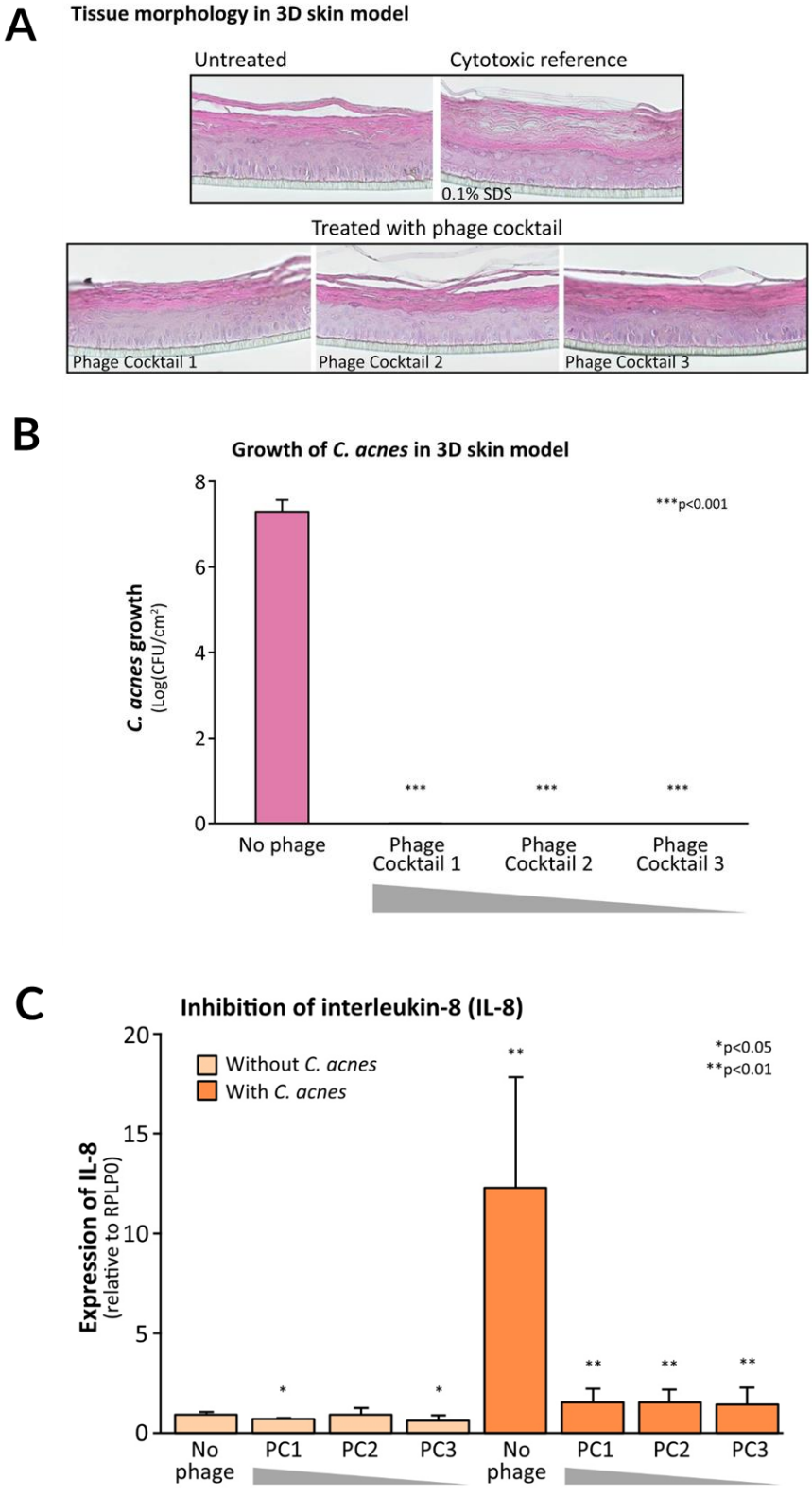
A
Tissue morphology in 3D skin model
Untreated
Cytotoxic reference
0.1% SDS
Treated with phage cocktail
Phage Cocktail 1
Phage Cocktail 2
Phage Cocktail 3
B
Growth of C. acnes in 3D skin model
***p<0.001
C. acnes growth
(Log(CFU/cm²))
8
6
4
2
0
***
***
***
No phage
Phage Cocktail 1
Phage Cocktail 2
Phage Cocktail 3
C
Inhibition of interleukin-8 (IL-8)
*p<0.05
**p<0.01
Without C. acnes
With C. acnes
Expression of IL-8
(relative to RPLP0)
20
15
10
5
0
No phage
PC1
PC2
PC3
No phage
PC1
PC2
PC3

Figure 3. A reconstituted human epidermal (RHE) model of blemish-prone skin corroborated the efficacy of the triple *C. acnes* bacteriophage combination as a solution to acne vulgaris **A.** The triple bacteriophage solution was examined for potential induction of detrimental histological alterations to the RHE of which none were detected except with a cytotoxic positive control indicating the bacteriophages are not noxious. **B.** After topically applying the bacteriophage combination to the RHE for 72 h, the tissue was swabbed and the material streaked on nutritive agar plates, incubated under anoxic conditions for 5 days, and subsequently examined for growth of *C. acnes*. In contrast to untreated tissue, little to no *C. acnes* growth was observed post-treatment. **C.** The RHE tissue was also tested for evidence of a reduction in the following inflammatory factors: IL-8, IL-1β, MIP-1β, and MCP-1. Only IL-8 (CXCL8 in graph above)) was shown to be dramatically reduced in response to the bacteriophages. NI: not inoculated. C: concentration (phage). Ca: *C. acnes* inoculated.

Cumulatively, the results of this study reinforced the phage-induced *C. acnes* clearance that was demonstrated under controlled conditions in bacterial cell cultures as well as the non-cytotoxicity of the combination. Furthermore, it also highlighted some anti-inflammatory potential also associated with the treatment. Thus, suggesting that phage therapy against *C. acnes* is an excellent candidate platform for an anti-acne regimen.

***Transmission electron microscopy identified the morphological lineage of the three phages investigated as modulators of the* C. acnes *residents on human skin*.** The individual phages that have been employed in combination to target *C. acnes* were examined for their morphological features by transmission electron microscopy (TEM). All three of the phages exhibited identical structural features consistent with members of the *Siphoviridae* family of bacterial viruses (Figure 4). These complex capsids possess a large icosahedral head of approximately 60 nm in width containing the linear, double-stranded deoxyribonucleic acid (dsDNA) virus genome, which is attached to a long tail that cannot contract unlike other bacteriophages. At the terminus of the tail structure are short fibers that facilitate attachment to the bacterial host cell. These phage structures that are identical those of the *Siphoviridae* family are consistent with what has been previously reported for the majority of phages that target *C. acnes* (20).

FIGURE 4

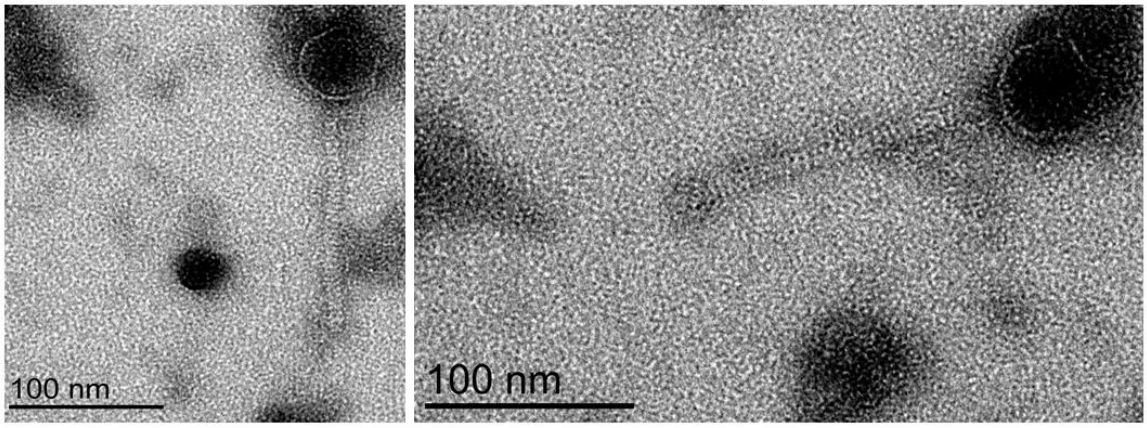

Figure 4. The triple bacteriophage combination was structurally evaluated by transmission electron microscopy. The capsid structures observed were consistent with other members of the *Siphoviridae* family of bacteriophages. This is consistent with the structures of all known bacteriophages that infect *C. acnes*.

The stability of the triple phage combination was evaluated in the presence of a variety of common skin care ingredients at different temperatures. As a novel bio-active ingredient for skin care regimens, it was important to investigate the compatibility of the *C. acnes* phage combination with several normal formulation components. To that end, the phage combination was rendered in a series of potential "carrier solutions" such that it could be added as a sub-phase to skin care products and evaluated for phage activity by downstream plaque assays.

In the first experiment, the phage mixture was rendered in 1,3-propanediol in an effort to prepare a preservative-free formulation. In the context of 100% 1,3-propanediol, all phage activity disappeared (data not shown). In parallel, aqueous carrier solutions containing either 0.7% phenoxyethanol or alternative preservatives such as 3% 1,2-alkanediol (Hydrolite-6®) with 1% hydroxyacetophenone (SymSave® H) were also tested. Unlike the outcome from 1,3-propanediol, water with phenoxyethanol and water with 1,2-alkanediol with hydroxyacetophenone had no negative impact on the phage combination to counter *C. acnes* and could be diluted to a significant extent (1:100,000 to 1:1,000,000) without loss (Figure 5). Additional solvents were subsequently investigated, including: 3% 1,2-pentanediol

(Hydrolite-5®) in water, 3% trehalose and 85% 1,3-propanediol in 12% water, 50% glycerin in water, and 100% butylene glycol. As shown in Figure 5, the dilution threshold for detection of lysis of *C. acnes* cultures by the phage combination fluctuated depending on which solvent system was employed. The data trends suggest that the phages function with greater efficiency when presented in solutions containing higher water content.

FIGURE 5

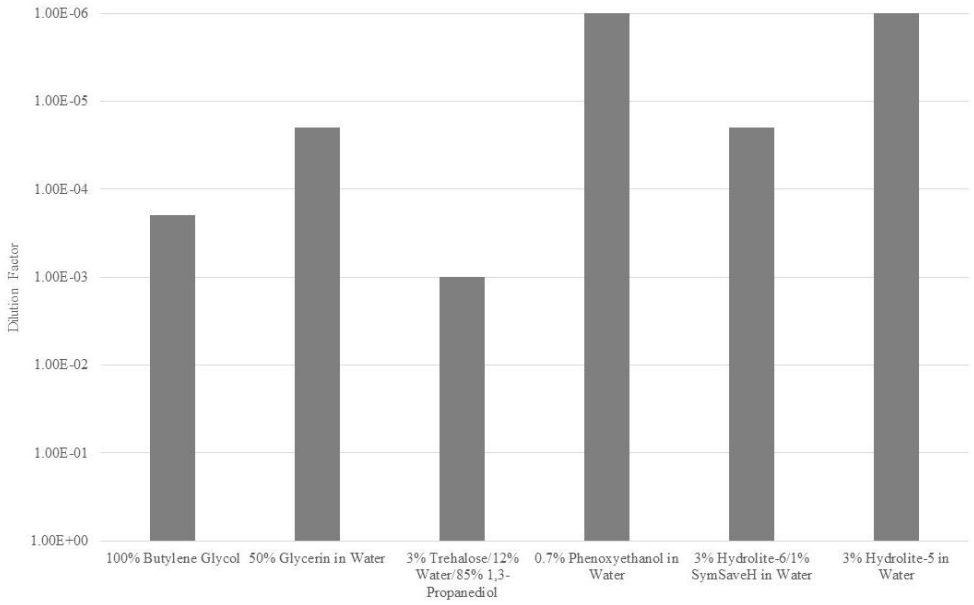

Figure 5. Triple bacteriophages targeted to *C. acnes* were mixed into a variety of carrier solutions to determine the phage compatibility with a variety of commonly employed cosmetic materials. Compatibility was assessed using a standard plaque assay to determine how far the phage could be diluted in the respective carriers.

The triple phage combination rapidly diminished C. acnes associated porphyrin fluorescence in a pilot clinical study. A pilot clinical study was commissioned to examine the potential reduction in porphyrin fluorescence associated with *C. acnes* growth on facial skin in response to application of a *C. acnes* phage topical formulation. Coproporphyrin III is the predominant porphyrin molecule produced by *C. acnes*, which produces a fluorescent pattern that can be monitored and correlated with the degree of bacterial infection. Here, a pilot group of 6 participants exhibiting degrees of acne vulgaris were examined for coproporphyrin III fluorescence at the start of the study and again after 7 days of twice daily

application of the triple *C. acnes* phage combination. Multiple high-resolution images were collected at a variety of camera angles (Figure 6). A noticeable reduction in coproporphyrin III fluorescence was observed, suggesting the efficacy of the treatment *in vivo* and that later time points would likely show a higher reduction in the fluorescence.

FIGURE 6

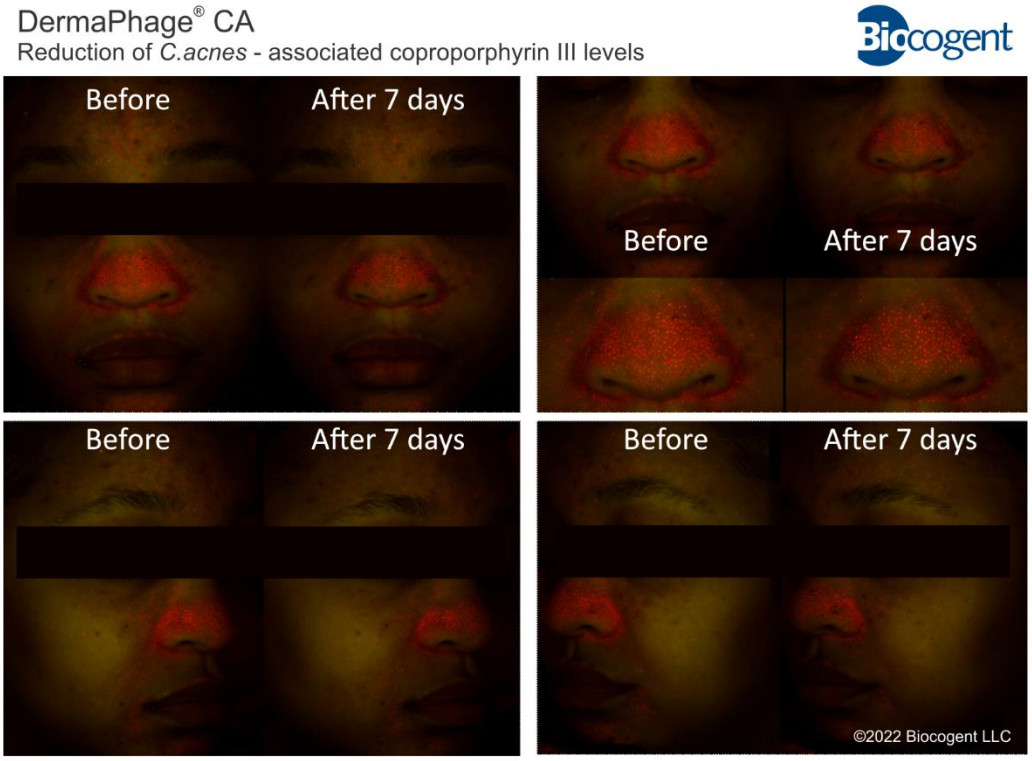

Figure 6. A pilot clinical study was commissioned to evaluate the efficaciousness of the triple bacteriophage regimen on a group of volunteers visualizing the rapid clearance of *C. acnes* by diminished coproporphyrin III-associated fluorescence levels. The pilot study consisted of 6 participants, all of whom reported improvement.

DISCUSSION.

Modern cosmetic care has initiated multiple campaigns to improve skin health through what are intended to be surgical modulations of the human skin microbiome. The precision being attempted is necessary given the growing appreciation of the impactful role played by normal commensal resident microbes in overall skin health. It is in this particular aspect that antibiotics have failed with their indiscriminate targeting of both beneficial and detrimental

microorganisms. Although certainly their activity decreases problematic populations, there is a collateral harm delivered upon the microbes that normally prevent pathogenic organisms from obtaining a foothold. Herein lies the strength of the phage therapy approach, where a single problematic bacterium is selectively diminished, leaving the other important skin microbial populations unaffected.

Acne vulgaris was chosen as the first skin affliction to be targeted in a proof-of-concept examination of phages modulating the skin microbiome. To that end, three unique naturally occurring phages were isolated from environmental samples that affected a lytic cycle in *C. acnes* – the bacterial species whose aberrant growth can contribute to incidences of blemished skin. Both the safety and efficacy of a triple phage combination targeted to *C. acnes* was repeatedly demonstrated using a variety of methodologies including *in vitro* and *ex vivo* evaluations as well as an *in vivo* pilot clinical study. In all instances, there was no detectable cytotoxicity inflicted upon human cells and tissues treated with the phages. Importantly, in all the experiments described for this study, the phages were highly effectual at substantially decreasing *C. acnes* levels with no apparent collateral damage to the rest of the skin microbiota.

The multiple approaches taken in this study further reinforce the contention that phage therapy is likely to be the successor of topical antibiotic regimens. The phage combination was highly effective at decreasing target microbial populations, but was also safe and fitting the current designation of "microbiome friendly". Therefore, it is reasonable to propose that applications of phage therapy will continue to be explored and applied by the cosmetic care industry to combat a variety of microbiome associated skin conditions.

CONCLUSION.

The findings of this investigation advance the hopes of modern skin care to devise ways of modulating the skin microbiome for ameliorative outcomes. In parallel, these efforts also champion the succession of phage therapy as the solution for the extinction of antibiotic effectiveness. Here, acnes vulgaris, the most common skin condition worldwide was targeted

in a proof-of-concept investigation of the utility of phage therapy to safely counter the *C. acnes*-based skin affliction. The successful outcomes of this study will fuel further expansion of phage therapy research in the cosmetic care industry.

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Conflict of Interest Statement. NONE.

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