Microbiological Insights and Dermatological Applications of Live Biotherapeutic Products

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Abstract

As our understanding of dermatological conditions advances, it becomes increasingly evident that traditional pharmaceutical interventions are not universally effective. The intricate balance of skin microbiota plays a pivotal role in the development of various skin conditions, prompting a growing interest in probiotics, or live biotherapeutic products (LBPs), as potential remedies. Specifically, the topical application of LBPs to modulate bacterial populations on the skin has emerged as a promising approach to alleviate symptoms associated with common skin conditions. This review considers LBPs and their application in addressing a wide spectrum of dermatological conditions with particular emphasis on three key areas: acne, atopic dermatitis, and wound healing. Within this context, the critical role of strain selection is presented as a pivotal factor in effectively managing these dermatological concerns. Additionally, the review considers formulation challenges associated with probiotic viability and proposes a personalised approach to facilitate compatibility with the skin’s unique microenvironment. This analysis offers valuable insights into the potential of LBPs in dermatological applications, underlining their promise in reshaping the landscape of dermatological treatments while acknowledging the hurdles that must be overcome to unlock their full potential.

Introduction

Our skin acts as an interface with the environment and is home to a delicately balanced and diverse microbial ecosystem, crucial in the maintenance of health and development of diseases (Skowron et al., 2021). This microbial ecosystem comprises a dynamic community of fungi, bacteria, viruses and archaea the abundance of which varies depending upon factors including pH (Proksch, 2018), salinity (Swaney et al., 2023), and exposure to environmental factors such as sunlight (Burns et al., 2019). Prevalent skin conditions including acne vulgaris and atopic dermatitis (AD) are characterised by perturbations in such communities, resulting in the overrepresentation of certain species within microbial consortia. For centuries, researchers and clinicians have attempted to address this imbalance through the application of antibiotics, which tend to eradicate all species rather than restore homeostasis. However, as we draw closer to the post-antibiotic era and our understanding of the
resident microbiome advances, novel agents capable of manipulating microbial communities towards homeostasis have proven increasingly attractive, with probiotics proving front runners in potential.

Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). However, as the scope and consumer interest in the potential therapeutic efficacy of these ‘medicinal microbes’ expanded, and the term began to lose specificity. Because only some countries regulate probiotics as drug products, many have not undergone clinical testing (Paraskevakos, 2022; Spacova et al., 2023). Probiotics are classed as food and supplements; therefore, they have not been widely adopted by the mainstream medical community for clinical use. Probiotics are instead endorsed for maintaining or improving a healthy state and are intended for use by healthy populations. However, the term probiotic is often used to describe any beneficial microorganism in a formulation, regardless of mechanism or clinical evidence supporting its efficacy (Cordaillat-Simmons et al., 2020).

Fundamental issues in experimental rigour and lack of regulation have resulted in the implementation of a new term; live biotherapeutic products (LBPs). Like probiotics, LBPs are living microorganisms, but rather than maintaining homeostasis, they are applied to prevent, treat, or cure a disease/condition, and may be taken by individuals afflicted with a particular condition (FDA, 2016). LBPs are a new category of medicines, officially recognised by FDA in 2012 and European Pharmacopoeia (Ph. Eur) in 2019, with stricter safety and efficacy guidelines that aim to legitimise and medicalise these treatments (Cordaillat-Simmons et al., 2020). LBPs are defined by as “medicinal products containing live micro-organisms (bacteria or yeasts) for human use” by the Ph. Eur, and this category does not include vaccines, gene therapy agents, or faecal microbiota transplants. As more products are tested and licensed, it is likely that new frameworks and policies will be implemented that clarify how the formulations containing microorganisms are presented.

While some probiotic strains are on the Qualified Presumption of Safety list or considered Generally Recognised as Safe (GRAS), requiring minimal pre-marketing approval, LBPs intended to treat, prevent, or cure diseases must undergo a rigorous pre-marketing approval process (Spacova et al.,
2023). In the US, the FDA requires potential LBPs to submit a Biologics License Application or New Drug Application, with specific guidelines covering chemistry, manufacturing, controls, preclinical, and clinical studies. The FDA adopts a product-specific approach to LBP development, given their novelty and diversity, but data must convincingly demonstrate the product’s quality, safety, and efficacy (Cordaillat-Simmons et al., 2020). Similarly, in Europe, LBPs must comply with EU legislation for biological medicinal products, requiring comprehensive data to obtain marketing authorisation. Companies can either apply for national authorisation in individual EU countries or opt for a centralised procedure involving multiple regulatory authorities (Franciosa et al., 2023). Marketing authorisation must also comply with consumer use, and according to the FDA, since LBPs are considered medicinal, their use must be restricted to food supplements, ingredients or drugs (Cordaillat-Simmons et al., 2020).

Many LBPs destroy pathogens using specific antimicrobial peptides (Nakatsuji et al., 2017) or non-specific compounds (Lebeer et al., 2022), others interfere with anti-inflammatory pathways in the host (Myles et al., 2020) (Figure 1). The history of modifying the gastrointestinal (GI) tract microbiome with probiotics is vast, and many studies exist examining the gut-skin axis (De Pessemier et al., 2021; Drago et al., 2014; Panduru et al., 2015). However, work on topical formulations of probiotics or LBPs for the skin is less examined. Topical application of next-generation LBPs could be used to restore the skin’s carefully balanced commensal community or introduce additional, advantageous microbes.

**Skin microbiome and LBPs**

While there is significant interpersonal and host-related variation in the skin microbiome, the most commonly identified phyla are Actinomycetotra, Bacteroidota, Bacillota, and Pseudomonadota (formally Actinobacteria, Bacteroides, Firmicutes, and Proteobacteria respectively) (Grice et al., 2009; Oren and Garrity, 2021). Composition and abundance depend on various local and holistic factors, such as skin dryness, sebaceous gland density, age, sex, and underlying medical conditions. Although these 4 phyla dominate, there is substantial variation at the species level. Skin is often
categorised into sebaceous (oily), moist, and dry areas, each with its own distinct community of native organisms (Costello et al., 2009). Sebaceous areas such as upper chest, back and facial folds are dominated by Propionibacterium spp. and have been shown to have the lowest phylotype and diversity richness, whereas moist areas are more populate with Corynebacteria spp and Staphylococcus spp. (Grice et al., 2009).

Resident microorganisms inhabit different depths within the skin layers. Aerobic bacteria are found at the surface, while anaerobic bacteria reside in the moderately anoxic microenvironments provided by the cutaneous compartments (Bay et al., 2020). The skin comprises a 50-70μm epidermal layer which is supported by a much thicker dermis below (4-5mm) and an adipose layer which provides insulation (Figure 2). The epidermis provides a physical and mechanical barrier to external stimuli and is formed almost exclusively of keratinocytes at different stages of differentiation. The surface layer of the epidermis, known as the stratum corneum, is composed of approximately 20-30 layers of dead, enucleated corneocytes, surrounded by a lipid matrix of cholesterol, free fatty acids and ceramides (van Smeden and Bouwstra, 2016). The lipid compounds in the stratum corneum are replenished by vesicles secreted from keratinocytes of the stratum granulosum, the layer below in the epidermis (Pouillot et al., 2008). Commensal bacterial species such as Corynebacterium, Staphylococcus, and Micrococcus utilise the epidermal lipids as a nutrient source or to produce anti-pathogenic fatty acids (Bomar et al., 2016; Zheng et al., 2022; Kengmo Tchoupa et al., 2023). Approximately 85% of bacteria reside within the top 6 layers of the corneocytes. However, the lower strata may not be devoid of resident microorganisms, as bacterial DNA remains detectable throughout the stratum corneum and even deep into superficial adipose tissue (Lange-Asschenfeldt et al., 2011; Nakatsuji et al., 2013).

Sebaceous glands are connected to hair follicles deep within the skin, providing a low-oxygen environment for commensal facultative anaerobes, such as Cutibacterium acnes (previously known as Propionibacterium acnes) and fermentative Staphylococcus spp. (Wang et al., 2014). The sebaceous glands secrete sebum, a waxy, lipid-rich substance that acts as a hydrophobic shield and first line of defence against invading pathogens. The composition of sebum is primarily triglycerides, wax esters,
and squalene. Microorganisms modify some of these compounds into free fatty acids that promote the adhesion of the resident symbiotic bacteria and reduce the local pH dissuading possible invaders (Skowron et al., 2021).

Disruption of skin barrier function can disturb the delicate stability of the skin microbiome. Damage may occur due to external stimuli such as UV or injury, allowing infection with opportunistic pathogens that can enter due to altered barrier integrity (Bowler et al., 2001; Patra et al., 2016). Internal pathologies like diabetes may increase the density of normally symbiotic microorganisms, resulting in an overgrowth of “contextual” pathogens (Gontcharova et al., 2010). Similarly, prevalent skin conditions including atopic dermatitis and acne vulgaris are often characterised by reduced microbiome diversity or overrepresentation of certain species within communities. Changes to microbiome composition observed range from an elevated abundance of Staphylococcus aureus in atopic dermatitis to an overrepresentation of specific C. acnes phylotypes in acne vulgaris, which is thought to contribute to disease pathogenesis and severity (Kong et al., 2012; Fitz-Gibbon et al., 2013; Spittaels et al., 2020; Conte et al., 2023; Deng et al., 2023). Microbiome composition is site-specific, therefore LBPs might differentially impact oily, dry and combination sites dependent upon microbial abundance and the presence or absence of certain species. Additionally, LBPs will target specific sites where disease often manifests. For example, acne vulgaris commonly impacts oily sites whilst atopic dermatitis impacts dry sites. As antibiotics and other treatments often do not improve long-term outcomes for patients with these common skin diseases, new approaches must be studied. Topical probiotics represent an attractive, non-invasive approach for the resolution of dysbiosis associated with prevalent skin conditions, with ever-mounting evidence supporting their use in the management of GI conditions.

**Acne**

Acne vulgaris (acne) is a highly prevalent condition, affecting up to 95% of the adolescent population (Silverberg and Silverberg, 2014). Acne has a multifactorial pathophysiology, involving androgenic stimulation leading to increased sebum production, hyperkeratinisation of follicle ducts, and
colonisation by disease-causing strains of *C. acnes*. This results in seborrhoea (itchy rash), pustules, papules (lesions) and comedones (blackheads), which in severe cases can result in extensive scarring and depigmentation of the skin (Silverberg and Silverberg, 2014).

Commonly prescribed topical medications for acne often contain a combination of a retinoid (like acitretin), and antimicrobials such as benzoyl peroxide and clindamycin (Zaenglein *et al.*, 2016). While moderately effective, some patients face challenges in tolerating these treatments due to skin irritation, resulting in discontinuation (Sevimli Dikicier, 2019). Alternatively, oral antibiotics and hormonal modulators can also be prescribed for acne but these interventions are associated with their own set of side effects (Baldwin, 2020). The use of antibiotics, particularly tetracycline and macrolides, to treat acne has faced criticism in recent years due to their role in contributing to antimicrobial resistance resulting in an increasing proportion of patients recalcitrant to mainstay treatments (Dessinioti and Katsambas, 2022; Leccia *et al.*, 2015).

There is ample evidence to support the role of *C. acnes* in the aetiology of acne vulgaris, but the mechanisms involved is still not fully understood (Dessinioti and Katsambas, 2017). This lipophilic, anaerobic bacterium thrives in the sebum-rich, anoxic environment created when sebaceous glands become obstructed. However, the relative abundance of *C. acnes* does not differ between affected and unaffected individuals (Fitz-Gibbon *et al.*, 2013). Of the three major divisions in the *C. acnes* species, studies suggest that only type 1 is involved in the aetiology of the skin condition, whereas types 2 and 3 are associated with healthy, non-acneic skin (Lomholt and Kilian, 2010). This is further broken down into subtypes with differing associations with skin disorders (Spittaels *et al.*, 2020).

In a small, open-label trial, two topical LBP formulations were evaluated in humans, each containing multiple (2 to 4) *C. acnes* strains (single locus sequence typing (SLST); C3, K8, A5 and F4) delivered in a hydrogel carrier. This study expanded on the theory that only particular strains of the bacteria are responsible for the skin disorder acne vulgaris, so beneficial strains were transplanted to reduce the population of the acne-causing organisms. Both formulations reduced the number of non-inflamed lesions (comedones) compared to the start of the study. The inflamed lesion count and skin pH were
not significantly different after treatment, but the overall \textit{C. acnes} proportion was higher compared to the baseline (Karoglan et al., 2019). This could suggest that modulation with \textit{C. acnes} is effective against the milder forms of acne, characterised by the presence of comedones that have not yet developed into inflammatory lesions or pustules. Conversely, Naked Biome has undertaken 2 clinical trials utilising proprietary \textit{C. acnes} strain NB01 as the medicinal product, but the results posted suggest that the intervention does not appear to be effective at achieving the primary outcomes (Naked Biome, Inc., 2020a, 2020b).

Interestingly, Paetzold et al. reported that the engraftment abilities of beneficial \textit{C. acnes} strains (SLST; H1, A1 and D1) were improved when applied in complex mixtures with other neutral strains, implying synergistic effects (Paetzold \textit{et al.}, 2019). The modulation of the \textit{C. acnes} population in this manner was successful in temporarily changing the abundance of strains present on the skin, and the duration of the abundance change was proportional to the dosage applied ($10^4$, $10^6$, and $10^8$ CFU ml$^{-1}$) as well as the diversity and number of \textit{C. acnes} strains applied to the skin. The success of engraftment was also dependent upon recipient microbiome composition, where individuals with higher levels of diversity initially proved more receptive to engraftment (Paetzold \textit{et al.}, 2019). However, following the cessation of treatment microbiome composition quickly returned to the ground state, owing to the temporal stability of the human skin microbiome (Oh \textit{et al.}, 2016). Though treatment of disease symptoms was not evaluated in this study, the application of \textit{C. acnes} was shown to be safe and effective (Paetzold \textit{et al.}, 2019). These studies highlight the need for more research into the delivery of a carefully selected consortium of health-associated \textit{C. acnes} phylotypes to target pathogenic \textit{C. acnes} strain.

As research into commensal strains has produced mixed results, the prospective efficacy of environmental strains derived from soil including \textit{Nitrosomonas} spp. including \textit{N. eutropha} have been investigated. The therapeutic action of this soil-dwelling microbe is thought to centre on its ability to reduce nitrate species to nitric oxide (NO). An increasing body of evidence points to the immunomodulatory properties of NO, through its ability to elevate the secretion of anti-inflammatory cytokine interleukin-10 (IL-10) and suppress the secretion of pro-inflammatory type 2 cytokines
including IL-4 (Maura et al., 2021). However, exposure to C. acnes is thought to elevate the secretion of Th17-associated cytokines, and how nitrate-reducing species manipulate these responses via elevated secretion of IL-10 remains to be characterised (Sardana and Verma, 2017).

Clinical trials have been completed by AOBiome utilising N. eutropha B244 (also known as D23) applied as a mist to the face and torso for the treatment of acne (Gryllos et al., 2016). The patients saw a significant reduction in the number of lesions and overall severity of acne at 12 weeks, but not at any previous time point analysed (AOBiome LLC, 2022a). This could suggest that LBP have a delayed effect, indicating that longer-term studies are needed. Interestingly, during the clinical trial for acne, a reduction in blood pressure was observed in participants. Elevated levels of circulating NO, produced by nitrate-reducing oral bacteria species, have previously been associated with lower blood pressure (Kapil et al., 2013). This outcome measure was monitored throughout the study to a build safety and tolerability profile for the strain and then made the focus of another clinical trial (AOBiome LLC, 2022b). Other studies by the same sponsors used N. eutropha B244 as a treatment for keratosis pilaris, a harmless condition where small bumps appear on the skin due to abnormal keratinisation of the follicular epithelium, and for reducing the visibility of wrinkles (Lee et al., 2018; Notay et al., 2020). This same strain used for multiple conditions suggests that the mechanism lies in the anti-inflammatory properties of ammonia oxidising bacteria (AOBs), rather than inhibiting or outcompeting the causative agents. However, caution should be taken when effects are modest, and local and systemic mechanisms are not clearly understood.

Recent microbiome studies have revealed a distinct gut microbiota in acne sufferers compared to healthy controls, indicating a strong gut-skin axis (Deng et al., 2018; Yan et al., 2018). Furthermore, case studies involving the oral administration of lactobacilli have demonstrated successful outcomes for acne treatment since the 1930s (Stokes and Pillsbury, 1930; Siver, 1961; Marchetti et al., 1987). Interventional human studies have also demonstrated that oral administration of Lactobacillus species is associated with improved symptoms (Fabbrocini et al., 2016; Jung et al., 2013; Rinaldi et al., 2022).
In vitro and explant studies indicate that lactic acid bacteria (LAB) could be applied topically for an effective, more targeted treatment (Khmaladze et al., 2019). The long-term usage, clear safety profile, and genetic knowledge of Lactobacillus species make them an attractive foundation for the development of a topical LBP (Lebeer et al., 2022). Espinoza-Monje et al. found that the topical administration of Weissella viridescens UCO-SMC3 (previously known as Lactobacillus viridescens) was more efficient than oral administration at reducing the C. acnes load of the skin. This bacterium was isolated from the slime of the common garden snail Helix aspersa Müller, and mucus from this species is known to have beneficial, cosmetic effects. Interestingly, the topical treatment with W. viridescens did not stimulate immune cells in the same way as the oral probiotics, suggesting that the effect is more localised. Furthermore, W. viridescens is effective at outcompeting C. acnes and S. aureus due to its noteworthy ability to adhere to keratinocytes. As no bacteriocin genes were identified, the authors propose that the bactericidal effect was likely due to lactic acid and H₂O₂, produced by many LAB (Espinoza-Monje et al., 2021). Unlike modulating certain strains of C. acnes, the mechanisms of inhibition proposed for LAB are non-specifically targeting susceptible strains, which may include acne-causing pathogens. An uncontrolled, preliminary clinical study also demonstrated that application of a formulation containing hyaluronic acid and Lactobacillus paracasei m.biome LiveSkin88TM DSM 33788 improved acne symptoms, leading to reduced pustulation and redness and an increase in skin hydration (Casari et al., 2022).

In a double-blind placebo-controlled clinical trial, Lactobacilli species were applied to the skin of acne sufferers and incorporated into an oil-in-water cream, also known as YUN ACN. The three species (L. pentosus KCA1, L. plantarum WCFS1, and L. rhamnosus GG) were chosen based on safety, robustness, and functional abilities to tackle an overgrowth of both acne-causing Staphylococcus and Cutibacterium species. The application of this LBP mediated an improvement in acne symptoms, reducing the number of inflammatory lesions and increasing moisturisation. The topical formulation decreased the relative abundance of Staphylococcus spp. without altering the diversity of the skin microbiome. The authors suggest that the Lactobacillus spp. skin supplementations had long-term immunomodulatory effects as improvements continued for more than
4 weeks after application. Interestingly there was no significant change in the number of non-inflamed lesions in this study (Lebeer et al., 2022). This observation raises the possibility that the grade/severity of the acne may need to be considered when choosing an LBP strain. It could be that the lactic acid/H₂O₂ are most effective on more pustulated acne. This is reasonable as a link between an increase in skin pH and acne has been established (Prakash et al., 2017). Future treatments may involve a combination approach, utilising C. acnes strains to combat comedones and a LAB to address deeper, more established infections.

*Atopic dermatitis*

Atopic dermatitis (AD), the most common form of eczema, is strongly associated with colonisation by *S. aureus* (Kong et al., 2012; Kolb and Ferrer-Bruker, 2024). A complex interaction between genetics and environmental factors can lead to dysregulation of the skin’s barrier function and a decrease in its antimicrobial abilities (Pessôa et al., 2023). These changes in skin conditions result in an increased pH and higher water evaporation, which can facilitate the overgrowth of *S. aureus* (Hülpschütz et al., 2020). During AD flares, *S. aureus* is overrepresented in the skin population, as the overall diversity of the microbiota is decreased. In one study, 10 of the 17 patients with AD did not have any culturable gram-negative bacteria (Myles et al., 2016). A “dose-dependent” response is seen as a higher population of *S. aureus* corresponds to more severe symptoms, possibly due to the expression of δ-toxin and phenol soluble modulin α (PSMα) toxin (Tauber et al., 2016; Nakatsuji and Gallo, 2019).

Many different organisms have been trialled for the treatment of AD, each with a different proposed mechanism of action. *Roseomonas mucosa* is a gram-negative skin commensal that has been studied for the treatment AD, demonstrated in both mouse models and as a first-in-human topical microbiome transplantation by Myles et al. (Myles et al., 2016, 2018). *R. mucosa* topically applied to the skin of AD-affected children and adults was correlated with clinical improvements and associated with a decreased *S. aureus* burden. The authors noted that this improvement was present even in AD patients who were already colonised with a different commensal *R. mucosa* strain. As with *C. acnes* treatment, the importance of strain selection is highlighted by this work (Myles et al., 2018). Evidence from the
groups’ mouse model studies suggested that only living *R. mucosa* is associated with improved clinical outcomes, whereas the application of lethally irradiated dead cells was not effective (Myles *et al.*, 2016). Subsequent work suggests that the bacterial production of sphingolipids, cholinergic signalling, and flagellin expression all mediate the cell-based host immune system, implying that the application of living *R. mucosa* may be essential. However, the authors do note that it may be possible to identify, extract, and formulate these lipids into stable topical formulations, but this has not yet been studied (Myles *et al.*, 2020).

Members from the same group were named as co-inventors on the patent for ‘Use of gram negative bacteria for the treatment of atopic dermatitis’ (US10195236B2), filed by the National Institute of Allergy and Infectious Disease (NIAID) and National Institutes of Health (NIH) (Myles and Datta, 2019). Product candidate FB-401, a topical spray which consisted of three therapeutic *R. mucosa* strains, was licensed and trialled by Forte Biosciences Inc (Forte Biosciences, Inc., 2021a). However, in a 16-week randomised, double-blind study on both adults and children, outcomes were not significantly different between treatment and placebo arms. The trial measured the proportion of subjects that had a 50% improvement in Eczema Area and Severity Index (EASI) score from baseline and found that 58% of subjects improved with FB-401 vs 60% in the placebo group (Priyan, 2023). As the primary objective was not achieved, further work with the product candidate was discontinued (Forte Biosciences, Inc., 2021b).

Various gram-positive species hold potential in the treatment of AD, with isolates of commensal, coagulase-negative staphylococci (CoNS), garnering particular interest. CoNS are known to produce bacteriocins, lantibiotics, and autoinducing peptides (AIP), which demonstrate selective inhibitory effects against *S. aureus*, sparing normal commensal and other *Staphylococcus* species. Building on a promising *in vivo* study (Nakatsuji *et al.*, 2017) and successful pilot trial (Nakatsuji *et al.*, 2021a), a randomised, double-blind, Phase I clinical trial conducted by Nakatsuji et al. and MatriSys Bioscience investigated the potential of *Staphylococcus hominis* A9 (MSB-0221) (Nakatsuji *et al.*, 2021b). The topical application of *S. hominis* A9 decreased the abundance of *S. aureus* on both lesional and non-lesional following 4 and 7 days of treatment. SCORAD results (a metric assessing global extent,
severity, and subjective symptoms) revealed a specific cohort of patients that exhibited positive responses to the candidate probiotic, relative to those receiving the placebo. Although all participants were initially culture-positive for *S. aureus*, the abundance of which is elevated in AD flares, the difference in response to the bacteriotherapy observed was linked to the lantibiotic sensitivity of *S. aureus* (Nakatsuji *et al.*, 2021b). As the authors suggest it is vital to reduce *S. aureus* numbers to improve symptoms of AD, this underscores the importance of understanding the sensitivity pattern of these organisms to prospective therapeutics. Importantly, *S. hominis* A9 also reduced expression of PSMα toxin, a major virulence factor secreted by *S. aureus* which contributes to inflammation associated with AD flare (Damour *et al.*, 2021). Inhibition of PSMζ was associated with an improvement in EASI (eczema severity) score regardless of lantibiotic sensitivity (Nakatsuji *et al.*, 2021b). Furthermore, *S. hominis* A9 produced AIPs, chemical signals secreted to manipulate the expression of virulence factors, biofilm formation and a host of other features that facilitate survival (Boles and Horswill, 2008). The authors found this mechanism of inhibition particularly interesting as resistance to these AIPs has not been reported (Nakatsuji *et al.*, 2021b).

Further work by the same group explored autologous transplantation of other CoNS as a strategy to overcome lantibiotic resistance. This study involved directly testing CoNS strains isolated from the AD patient for their inhibition of *S. aureus* isolated from the same individual. This personalised matching of LBPs led to a significant reduction in the *S. aureus* population on the skin at days 4, 7 and 11 of the study. However, the improvement in EASI score wasn’t significant until day 11 (Nakatsuji *et al.*, 2021a). This could indicate delayed positive outcomes that could not be observed in the short 11-day study, a phenomenon also observed in the AOBiome study using *N. eutropha* B244 for the treatment of acne (AOBiome LLC, 2017). The intricate logistics of identifying and culturing personalised strains for this tailored treatment were not discussed by the authors (Nakatsuji *et al.*, 2021a). Other *S. hominis* strains have been studied for their safety and antibiotic sensitivity profile (Mozyrska *et al.*, 2023). Collectively, these studies demonstrate the safety and efficacy of topical application of CoNS (particularly *S. hominis*), setting the foundations for longer trials. MatriSys Bioscience have announced a phase II proof-of-concept study for *S. hominis* A9 (MSB-0221) starting
in 2023, alongside an ongoing Phase Ib pharmacokinetics/pharmacodynamic study sponsored by NIAID (“MatriSys Bioscience Announces Corporate and Strategic Update,” 2023). As these commensal organisms produce compounds that effectively inhibit their frequently encountered, potentially pathogenic neighbours, other commensal organisms probably possess similar antimicrobial and anti-inflammatory properties.

The soil microbe, *Janthinobacterium lividum* (referred to as DBI-001) exhibited safety, tolerability, and effectiveness in reducing *S. aureus* for the treatment AD, in a randomised phase IIa trial, but no mechanism has been revealed (DermBiont, Inc., 2021). Similarly, *N. eutropha* B244 applied directly to the face in a spray has shown effectiveness at treating mild-to-moderate AD in adults. In a phase IIb, double-blind clinical trial, the topical application of the ammonia-oxidising bacteria was significantly better than the vehicle alone at reducing eczema area and severity (Silverberg *et al.*, 2023). This study showed that itching intensity decreased as the patients underwent treatment. Although the principal mechanism theorised was the production of anti-inflammatory nitrite compounds, the authors suggested that the formulation might also reduce the *S. aureus* populations, as previous *in vitro* experiments had demonstrated (Gryllos *et al.*, 2014). However, the microbial population was not examined in the human studies (Silverberg *et al.*, 2023).

Alongside the treatment of acne vulgaris, the prospective efficacy of Lactobacillus for the management of AD has also been investigated (Lebeer *et al.*, 2022). Many species of Lactobacillus show inhibition of AD-related strains of *S. aureus*, thought to be due to their ability to lower the pH of the local environment (Christensen *et al.*, 2021). The multi-strain YUN cream, previously discussed in the context of treating acne, has undergone a clinical trial for remedying AD, but no results have yet been posted. Butler *et al.* with BioGaia formulated live *L. reuteri* DSM 17938 into an oil-based product containing shea butter and canola oil for application to patients affected with AD. A proof-of-concept study reported the ointment significantly improvement of the SCORAD index after 4 and 8 weeks of use, reduced itching and improved sleep. However, there was no significant difference in any beneficial outcome when compared to the placebo, vehicle-only control. This suggests that enhanced hydration of the skin or other chemical mechanisms is mostly responsible for improved
clinical outcomes. (Butler et al., 2020). A similar Lactobacillus study claims that L. sakei probio 65 incorporated into an emollient (moisturiser) had improved outcomes compared to the emollient only, in a split body clinical trial (Park et al., 2014). Neither of these studies strongly suggests Lactobacillus treatments provide superior outcomes compared to the vehicle controls. If the predominant mechanism of action against AD and S. aureus is the production of acidic substances, then it may be that this is neutralised by the moisturiser components.

Despite the strong correlation between abundance and symptom severity, treatments of AD using Staphylococcus, Roseomonas, and Nitrosomonas spp. all suggest that directly reducing S. aureus numbers is only a single feature of successful LBPs. These studies indicate that more research is needed to understand the underlying mechanisms behind AD. As the studies show many independent mechanisms, it is likely that AD treatment can be approached from different directions.

Fungal infections

Promising results from a phase IIb trial were reported for treatment of the fungal foot infection tinea pedis (athlete’s foot) using a previously mentioned species J. lividum (DBI-001), although full results were not released (DermBiont, Inc., 2019). A further unidentified strain DBI-002 has been trialled for the treatment of another fungal skin infection, tinea versicolor, and in combination with BDI-001 for tinea pedis (DermBiont, Inc., 2023). However, DermBiont are apparently not currently expanding work on these products, citing the termination of clinical trials during the COVID-19 pandemic and lack of investor interest (LaHucik, 2023). Despite promising in vitro work, no more in vivo studies are currently published on the use of LBPs for the treatment of fungal infections (Wu et al., 2022).

Skin wound healing including burns and diabetic foot ulcers

When the skin barrier is compromised due to injury it is left susceptible to infection. Healing of skin wounds can be complicated as infections with S. aureus, beta-haemolytic Streptococci and Pseudomonas aeruginosa are common (Bowler et al., 2001). The destruction of the skin barrier and normal microbiota enables these opportunistic or pathogenic microorganisms to establish highly
resistant biofilms that are characteristic of >80% of chronic wounds (Cámara et al., 2022). The proliferation and spread of these organisms can result in dire consequences such as sepsis and death (Peral et al., 2009). It has long been suggested that a microbial imbalance can lead to prolonged wound healing times, so it is plausible that supplementation with living microorganisms may help (Robson, 1997).

Given that in these cases the probiotic is directly applied to an exposed wound area, the potential for systemic infection is greater than treating other conditions. Consequently, the strains selected are subjected to strict safety standards, ensuring they exhibit no pathogenicity. Therefore, the extensively investigated LAB have predominantly been studied. LAB are ubiquitous and alongside lactic acid production, they also synthesise several immune-modulating compounds (Brandi et al., 2020). In vitro L. plantarum ATCC 10241 interrupts P. aeruginosa PA100 biofilm and elastase formation, and inhibits growth by interfering with quorum sensing molecules (Valdéz et al., 2005). Many animal studies have suggested that Lactobacillus spp. (especially L. plantarum) are safe, and inhibit the growth of common pathogens whilst improving tissue repair (Argenta et al., 2016; Sürmeli et al., 2019; Valdéz et al., 2005).

In an early example of successful bacteriotherapy, Peral et al. found that direct topical application of L. plantarum ATCC 10241 cells (in MRS growth media) to burnt areas had comparable results to the gold standard treatment, silver sulphadiazine (Peral et al., 2009). Moreover, another uncontrolled trial in burn patients showed that the application of the same L. plantarum strain reduced the count of pathogenic organisms and decreased apoptosis and necrosis of neutrophils by an IL-8-dependent mechanism (Peral et al., 2010). A larger follow-up study compared healing after surgical debridement of chronic diabetic foot ulcers, with and without the addition of L. plantarum ATCC 10241. Again, the cells were added directly to the wound area, reasoning that this treatment would be used as an economical treatment in the hospital environment. The addition of this LBP significantly reduced microbial load at days 14 and 18, reducing the chance of pathogenic invasion. The therapy also decreased the wound size significantly quicker than surgical debridement, with 70% of patients achieving complete wound closure by day 60 (vs. 40% in the control group) (Argañaraz Aybar et al.,
2022). A case report also outlines the application of a multi-strain probiotic product (including *L. plantarum* NCIMB 43029, *Lactobacillus acidophilus* NCIMB 43030 and *Streptococcus thermophilus* NCIMB 30438) directly to a non-healing wound as a last resort. The probiotic eliminated multidrug-resistant strains of *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Proteus mirabilis* within 21 days, after antibiotic therapy had failed (Venosi *et al*., 2019). These positive outcomes provide good evidence that topical application of *L. plantarum* is effective at improving outcomes for burns and wound healing. However, larger clinical trials are required before integration into clinical practice.

Patches made of collagen, dressings, or other biomaterials, are an explored method for delivering probiotic organisms to wounded areas. This is because they can be applied directly to an affected area and left *in situ* for the duration, whilst the microbes can replicate on the patch where they may produce nitric oxide or other anti-inflammatory compounds (Jones *et al*., 2012). As an alternative to a conventional dressing, the patch retains moisture and stops the wound from drying out (Oryan *et al*., 2018).

In a unique example with a distinctive mechanism, microalgae *Chlamydomonas reinhardtii* cw15-30-derived UVM4 were cultivated on a scaffold with human fibrinogen and thrombin, resulting in an insoluble, photosynthetic patch. The patch was subsequently applied to chronic wounds in humans, and light was applied. The oxygen generated through algal photosynthesis promoted healing, akin to the effects observed with hypobaric therapy. Notably, it addresses the anoxic environment, a significant factor in chronic wounds, and focuses on healing the host cells without directly targeting the bacterial pathogens. The microalgal cells were present in the tissue at day 14, suggesting movement out of the patch and into the wound area. The authors emphasise that the absence of immune-stimulating lipopolysaccharides on the surface of the eukaryotic *C. reinhardtii* cells renders them safe for humans and well-suited for oxygen-releasing patches (Obaid *et al*., 2021). In an *ex vivo* skin study, *Saccharomyces cerevisiae* was infused with curcumin and then applied to skin samples, demonstrating the concept of using yeast cells as a drug delivery method (Dou *et al*., 2021). Animal studies suggest that yeasts can enhance the healing of wounds (Oryan *et al*., 2018; Partlow *et al*., 2016), but this is an underexplored area of research as few other eukaryotic organisms are currently
being tested on humans for any dermatological conditions. Furthermore, these cells could also be engineered to deliver immune-stimulating and/or wound healing substances. Eukaryotic organisms can also be genetically engineered to produce complex human recombinant proteins that may not be translatable in prokaryotic systems (Müller et al., 2006).

Genetically Modified Probiotics

As knowledge of bacterial physiology and synthetic biology progresses, there is now the potential to genetically manipulate microbes to produce active products on the skin. Success has been seen in animal studies trialling next-generation, genetically modified (GM) LBPs for improving the healing of wounds. For example, Vågesjö et al. engineered *Limosilactobacillus reuteri* to express the chemokine CXCL12 (stromal cell-derived factor 1α), a protein associated with beneficial healing effects in models of cutaneous wounds. Topical application of GM *L. reuteri* led to the on-site microbial production of CXCL12, and shortened the healing period in diabetic mice, and mice with peripheral hind limb ischemia (Vågesjö et al., 2018). Further work by the same group found similarly accelerated healing of full-thickness wounds in mini pigs (Öhnstedt et al., 2022), and the research is progressing into phase II clinical trials in Europe (European Union Clinical Trials Register, 2024; Ilya Pharma, 2024). Other work with genetically engineered *Lactococcus lactis* subsp. *cremoris* MG1363 has demonstrated that the GM cells can express multiple therapeutic proteins designed to target various aspects of wound healing. The plasmid-carrying bacteria produced 3 different proteins; human fibroblast growth factor 2, IL-4 and colony-stimulating factor 1, that act synergistically to regulate the immune response and promote wound healing (Kurkipuro et al., 2022). In another study, recombinant *L. lactis* expressing the mucosal protectant human trefoil factor 1 (hTFF1), was rinsed around the oral cavity, producing the compounds *in situ* (Limaye et al., 2013)

Using the newly expanding genetical manipulation toolkit for *Staphylococcus* spp., it is possible to introduce new genes into the organisms, such as a biosensor that can detect and react to the presence of *S. aureus* quorum sensing molecules that may be beneficial in the treatment of AD. In the presence of these proteins, the engineered *S. epidermidis* ATCC12228 cells produced antimicrobial
compounds. This genetic switch reportedly reduces autotoxicity and fitness issues in the expression strain, but also protects the native microbiota from overexposure to antimicrobials (Guan et al., 2022). As CoNS including *S. epidermidis* have many mechanisms of inhibitions and numerous cell-host effects, they are an ideal starting point now modern synthetic biology tools are available.

*S. epidermidis* strains promote skin barrier maintenance by converting sphingomyelin into ceramides and phosphocholine. These ceramides contribute to the retention of water in the skin, and a reduction is associated with increased symptoms of AD (Zheng et al., 2022). Phosphocholine utilised as an energy source or a precursor to an osmoprotectant crucial for survival on the salty skin (Geiger et al., 2013). The sph gene responsible for the sphingomyelinase was overexpressed in the heterologous host *Staphylococcus carnosus* TM300 to confirm the involvement and safety of the enzyme. However, the authors did not test this GM bacteria directly on skin as *S. carnosus* is not a skin commensal and does not have the other benefits demonstrated by *S. epidermidis*. The authors suggest selecting *S. epidermidis* strains with high Sph activity, but this single gene manipulation seems an ideal target for genetic modifications (Zheng et al., 2022). As this manipulation involved only altering the expression patterns of autogenous genes and does not introduce exogenous DNA, this may be a more acceptable form of GM organisms with a higher probability of passing regulatory standards and gaining wider public acceptance.

The safety aspects of GM organisms must be considered when modulating the microbiome (Kurkipuro et al., 2022). Dodds et al. demonstrated alanine auxotrophy to control the growth of transplanted CoNS, *S. epidermidis*. In GM cells, genes required for D-alanine synthesis from L-alanine and D-glutamate were knocked out, meaning supplementation with D-alanine was required for growth. As alanine is not produced by keratinocytes, this mutation could be integrated into other GM probiotics, where any growth medium or formulation would be the sole source of the amino acid. Discontinuing feeding would disable cells, acting as an effective control method (Dodds et al., 2023).

This technology is under phases I and II study by Azitra Inc, for the treatment of papulopustular rash that arises due to cancer treatment that is associated with *S. aureus* overgrowth. Other GM strains of
S. epidermidis are being investigated by the same group for the treatment of Netherton syndrome and ichthyosis vulgaris (Azitra Inc, 2023).

Other commensal organisms have also been genetically modified to improve the safety of the product. To control growth, a genetic switch was introduced into C. acnes subspecies defendens (a ribotype associated with healthy skin). An arabinose inducible promoter was placed in front of the ftsAZ operon, controlling the expression of the genes required for cell division and ensuring that the bacteria could not replicate without the addition of the sugar. The cells remained viable but non-replicating on the skin but could be revived by adding arabinose any time up to 30 days. This system was reported to reduce the proliferation and avoid unintended transfer to other skin areas or people (Rhee et al., 2023).

Clinical trials

Topical LBPs are a hot topic in this developing subject area. For the past decade, human clinical trials have taken place testing the application of active cells to the skin, the outcomes of which are summarised in Table 1. These trials mainly focus on the treatment of dermatological diseases by reducing pathogen load and/or modulating local inflammation. Most trials were registered as Phase I and Phase II to establish safety and efficacy, but AOBiome has completed a Phase III study for the treatment of acne using a propriety soil bacteria strain. There is also a phase III study registered in the Iranian clinical trial registry using L. reuteri JCM 1112c for AD, but results have not yet been reported. Both acne and AD are the most targeted conditions, with several studies utilising a range of bacterial species. Often the same probiotic strain has been trialled for several conditions. Treatments for other conditions have been studied, including fungal skin conditions (tinea pedis and tinea versicolor) and for promoting wound healing of diabetic ulcers. Generally, the microbes studied are skin commensals, exogenous soil organisms, or the widely used lactic acid bacteria. A small number of studies investigated the use of genetically modified organisms, although this is a promising area that is of significant interest. However, despite growing evidence of clinical improvement following
exposure to probiotics, several studies have reported that placebo had comparable effectiveness to the probiotic in mitigating symptoms.

**Next-generation dermal biotherapeutics?**

Despite making up around 25% of species on the skin, no clinical studies exist using Corynebacterium species as an LBP, but manipulation of Corynebacterium abundance has been proposed for the management of armpit odour (Callewaert, Lambert and Van de Wiele, 2017). As a commensal of the skin shown to be involved in lipid breakdown and replacement, it may be a target organism for future therapies. For example, the application of Corynebacterium spp. to the airways of mice was successful in preventing infection with S. pneumoniae by producing triolein, a triglyceride shown to inhibit certain pathogenic bacteria (Horn et al., 2022). However, it is unlikely that this lipid-dependent mechanism of inhibition would control the growth of S. aureus or C. acnes (causative agents in acne and AD) as they both hydrolyse triolein (Dougherty and McCulley, 1986). Other mechanisms of inhibition have been suggested, such as the interaction between S. aureus and Corynebacterium striatum through quorum-sensing peptides. These peptides reduce the virulence of pathogenic S. aureus returning them to a commensal state, and promote adhesion of the microbial cells to the keratinocytes (Ramsey et al., 2016). Although the relationship between bacteria on the skin is not well understood, Corynebacterium have adapted to their niches to form a stable population, possibly using antimicrobial substances to defend their environment (Kwaszewska and Szewczyk, 2007). In future, these strains may be exploited for the treatment of AD or acne.

All previously mentioned mechanisms have involved one species of microorganism producing antimicrobial compounds to combat competitive pathogens. Although the inhibitory substances produced vary, the pathogens may develop resistance to many of these mechanisms. On the other hand, obligate predatory bacteria, such Bdellovibrio and like organisms (BALOs) attach to and physically enter their prey microorganism. After replicating, the BALOs enter the attack phase and the progeny burst out, lysing the host cells. BALOs are known to selectively prey on a range of gram-negative bacteria (including multi-drug resistant organisms) and as they are obligate intracellular
predators, there would be little concern about resistance mechanisms or biocontainment. Facultative predators also exist that can behave saprophytically and secrete extracellular antimicrobial compounds, or instead replicate inside prey organisms (Bratanis et al., 2020). Predatory BALOs have shown to be effective against several gram-negative species and their pre-existing biofilms, but their impact on skin pathogens has not been established. No reported predatory bacteria can prey upon gram-positive species (Waso-Reyneke et al., 2022), but there is evidence suggesting that extracellular serine proteases from B. bacteriovorus can degrade and inhibit the formation of S. aureus biofilms (Monnappa et al., 2014).

Due to their specificity targeting gram-negative bacteria, these organisms would be better suited to treating infected wounds and burns (Waso-Reyneke et al., 2022). This has been safely demonstrated by Tajabadi et al., where Bdellovibrio bacteriovorus HD100 was applied as live antibiotic to mice to treat burns inoculated with P. aeruginosa. This treatment significantly reduced the P. aeruginosa burden compared to the control, and better than the standard antibiotic treatment at 3 out of 4 timepoints (days 3, 14 and 22). The authors also report that the predatory bacteria accelerate the healing rate, by modulating inflammatory mediators and promoting collagen synthesis (Tajabadi et al., 2023). Furthermore, these bacteria have been formulated into a poly(vinyl alcohol) (PVA)/alginate hydrogel that showed improved microbial stability exhibiting similarly improved wound healing (Liu et al., 2023b).

Formulation

As this area of research is reasonably recent, the greatest area of interest has been the selection of safe and effective microbial species for the treatment or prevention of disease. Despite some promising evidence in that area, there is comparatively little published data on the effect of formulation on storage, delivery, or colonisation of skin by topically applied LBPs. The formulations vary between studies, subjecting the bacteria to different environmental conditions and processes. Various studies exist on the formulation of these microorganisms into a deliverable drug product, but very little
comparing the different stabilisation methods and their effect on the microorganism and/or clinical outcomes.

Although the viability of cells during formulation and storage is sometimes monitored, it is often overlooked. Where studies do exist, heterogeneity among incorporated species and testing methodologies makes comparison of techniques difficult. Formulation for the skin has different considerations compared to delivering the LBP orally for colonisation of the GI tract. Whereas orally consumed probiotics must survive the acidic conditions of the stomach, the challenge with topically applied microorganisms is maintaining their stability in a skin-safe formulation. Even formulation of lysate or bioactive compound-containing creams has been reported as difficult, for example, a sonicated *S. thermophilus* based cream had issues with stability and unpleasant sensory features after several weeks, even when refrigerated (Zoccali et al., 2016).

Various factors such as temperature and oxygen exposure during processing and storage can affect the viability of microorganisms during processing (Santivarangkna et al., 2007). Although many topical formulations contain aerobic organisms, important organisms such as *C. acnes* and *Lactobacillus* species are anaerobic bacteria with varying oxygen tolerances that can sustain reactive oxygen species-related damage during stabilisation processes. Furthermore, most liquid or frozen probiotic cultures require refrigeration for storage and distribution, thereby adding expense and inconvenience.

The choice of base carrier must also be considered. As patients with the mentioned dermatological conditions often have weakened barrier integrity, it is paramount to have a skin-safe, non-irritating cream or ointment. Repeatedly it has been seen that the vehicle control performs just as well as the probiotic-containing drug substance in outcome measures (Ben David et al., 2021; Parveen et al., 2023). As the formulations are often a designed and studied cosmetic product, it is unsurprising that these have beneficial effects on the skin. It must be considered that the formulation of the vehicle itself is a compounding factor and may be the principal mechanism for improvement.
Dosing

Once manufactured into a product, the formulation must support a suitable count of viable cells in an acceptable physiological condition for the intended storage period. This formulation must then deliver a large enough population of living cells to the skin to elicit a reproducible response in the patient.

Although dosing studies exist for GI probiotics, there is not a proportional dose–effect relationship as expected, meaning at higher doses the outcome is not necessarily more successful (Pot and Vandenplas, 2021). In existing topical studies, cell number per dose is variable across studies, as are the dosage schedules. A study with *R. mucosa* used an escalating dosing regimen, starting with $10^3$ cells and incrementally increasing to $10^5$ cells applied topically twice per week (Myles et al., 2018).

The inoculation schedule may be specific to the bacteria, for example, slow growing *R. mucosa* cells were only applied twice weekly, allowing time for the cells to colonise and reach the stationary phase before new cells were applied. (Myles et al., 2018). There is a suggestion that the dosing schedule depends on the anticipated mode of action, but this has not been explored for skin LBPs (Pot and Vandenplas, 2021). Furthermore, it has been suggested that the host specificity of the probiotic reduces the accuracy of animal models and limits their value in determining dose regimens for human studies (Ducarmon et al., 2021).

Whereas Karoglan et al, topically applied $10^6$ total *C. acnes* cells twice daily (Karoglan et al., 2019), Paetzold et al, investigated different dosing values at $10^4$, $10^6$, and $10^8$ CFU ml$^{-1}$ and higher concentrations showed greater engraftment (Paetzold et al., 2019). This dose-dependent response implies that application of *C. acnes* is more effective when applied at higher concentrations. However, since both studies used previously unstudied commensal bacteria, it is not possible to determine the extent to which strain-dependent effects influence the results. In their pilot study, Nakatsuji et al applied $1 \times 10^6$ CFU *S. hominis* ShA9 cells per cm$^2$ to the skin twice daily (Nakatsuji et al., 2021b), and in a later study used $2 \times 10^7$ applied to a whole arm (Nakatsuji et al., 2021a). As there are approximately $10^4$ to $10^6$ bacteria per cm$^2$ of skin, these studies suggest that it is necessary to outnumber the local microbiome to elicit a beneficial effect (Smythe and Wilkinson, 2023).
Other studies use microorganisms in higher numbers and more frequent schedules similar to those used in GI products. For example, for treatment of acne patients were asked to apply $10^8$ CFU *Lactobacillus* cells to the face twice daily (Lebeer *et al.*, 2022). Genetically engineered *L. reuteri* strain ILP100 was tested at 3 different doses ($10^5$, $10^7$, and $10^9$ CFU cm$^{-1}$), and the cohort receiving the highest dose showed the greatest improvement in healing time (Öhnstedt *et al.*, 2023). Similarly, *N. eutropha* B244 was applied at a comparatively high dose of approximately $10^9$ cells per application for the treatment of AD (Silverberg *et al.*, 2023) and wrinkles (Notay *et al.*, 2020), but another paper using this organism for keratosis pilaris did not report a dosage (Lee *et al.*, 2018).

In some studies, there is a lack of monitoring or reporting regarding the survival of the probiotics in the formulations before application, potentially meaning the vehicle may not be delivering live cells to the skin. Whilst there is evidence that the remaining cell components may have a therapeutic effect, colonisation of the skin is frequently a primary objective as well. Where survival statistics are provided, there is great variety in the bacterial species and compounds used in the formulations which makes it difficult to make comparisons between studies. It is clear that more time needs to be spent confirming that the cells have the ability to remain viable, and to ensure that a consistent, reproducible number of beneficial microorganisms is being delivered to the patient in both clinical trials, and in future prescribed products (Fiore *et al.*, 2020).

**Freshly cultured microbial cells**

Due to the novelty of the research and the small scale of trials, some of the probiotics are delivered completely unformulated. In these cases, freshly cultured cells may be taken directly from the laboratory and immediately applied to a treatment area. Peral *et al.* directly applied *L. plantarum* ATCC 10241 cells in MRS growth media to burn areas, where whole cells and extracellular metabolites present in the broth to the wounds promoted granulation and healing, whilst simultaneously reducing the local bacterial load. The authors suggest that this may be due *L. plantarum* instigating the induction of cytokines in immune cells that counteract those induced by the pathogenic *P. aeruginosa*. However, they accede that the beneficial effect could also be due to the
chemical properties of the supernatant (Peral et al., 2009). Delivery by this rudimentary method is impractical at scale and introduces several quality and safety considerations, but is an ideal, low-cost treatment for in situ treatment of stubborn, non-healing wounds in a clinical setting.

In simplistic formulations, the cells may be washed and resuspended in a neutral liquid matrix solution, such as peptone water, in which C. acnes strains from healthy volunteers was reported to be stable for 6 weeks at room temperature (Paetzold et al., 2019). Preservation of C. acnes in other aqueous solutions such as 20% ethanol and 20% propylene glycol was moderately successful for 6 weeks, though a longer shelf-life would be needed for dispensing as a pharmaceutical product (Carbol et al., 2018).

In clinical studies trials by AOBiome, N. eutropha B244 was reconstituted in a simple aqueous buffer (Na₂HPO₄ and MgCl₂) at the clinic and then taken home by the patient for administration over 10 days (Silverberg et al., 2023). Other literature suggests that N. eutropha D23 is stable at 4°C for several months (Gryllos et al., 2014), and similar AOB can withstand periods of starvation in nature (Geets et al., 2006). In this situation, the strain choice allows for a simplistic but effective preservative and delivery method, that would not be suitable for other, less resilient microbes.

Ideally, the beneficial microbe would be delivered in a vehicle that also promoted skin healing, as improperly formulated moisturisers can cause further symptoms and irritation to AD patients (Elias, 2022). Moisturisers are enriched with occlusive ingredients like petrolatum or lanolin to repel water, balanced with a humectant like glycerol to trap moisture in the stratum corneum. They also often include vegetable oils, some of which are rich in fatty acids that may improve barrier function and reduce inflammation of the skin (Elias, 2022). In work using S. hominis A9 and CoNS by Nakatsuji, the freshly cultured probiotic was resuspended in 50% Cetaphil lotion, 50% cosmetic-grade vegetable glycerol, and frozen at -80°C until dispensed. The glycerol component may act as a cryoprotectant and the lotion as a safe vehicle for delivering onto the skin of the AD patient (Nakatsuji et al., 2021a, 2021b). This method is well suited for generating personalised CoNS strains for brief trials.
Nevertheless, the stability of these strains might be poorer at elevated storage temperatures, particularly during transport and at the patient’s home.

Microencapsulation techniques such as extrusion and emulsification are proposed but have not gained traction in pharmaceutical manufacturing. Both processes often involve the resuspension of fresh probiotic cells in an aqueous solution containing a polymer such as alginate, carrageenan, and pectin. In emulsification, this solution is then dispersed within a lipid base, often a vegetable oil. Subsequent gel formation is achieved by the dropwise addition of divalent cations in the form of calcium chloride, encapsulating the microorganisms in a bead. During extrusion, the bacteria-polymer mixture is dropped or sprayed to a solution of calcium chloride, forming polymer beads around the microorganisms without the lipid component (Burgain et al., 2011). Extrusion has been used to formulate a novel antibiotic-probiotic mix that can be directly applied to the skin to treat complex polymicrobial or multidrug-resistant pathogens (Li et al., 2018). The beads produced by encapsulation are often lyophilised, frozen, or incorporated into a further formulation for application.

Spray drying is a semi-standard method for stabilising microorganisms that have seldom been used for topical probiotics, likely due to the risk of thermally deactivating the bacteria. However, David et al. spray-dried Bacillus subtilis NCIB 3610 cells in a protective 2.5% aqueous PVA solution and the collected microparticles were applied directly to a wounded mouse to examine healing. The B. subtilis in the particles did reduce the relative wound area faster than the untreated control, but not significantly better than the empty particles, suggesting that PVA may be having the larger effect (Ben David et al., 2021).

Inspired by biofilms, hydrogels incorporated into wound dressings have gained popularity as a research focus. These hydrogels are proposed for wound and burn care, as the matrix can cover a large surface and be left in place for a prolonged period. Initially, the probiotic cells are contained within the gels, which provide an acceptable habitat with access to nutrients. Unlike direct application to the skin, this approach allows probiotic microorganisms to potentially avoid direct competition with the skin’s microbiota for resources. Instead, the organisms colonise the wound dressing
hydrogel, establishing a community of active cells capable of releasing anti-inflammatory metabolites onto the skin, or colonising it once a quorum has been established (Liu et al., 2023a). It has also been suggested that certain probiotic hydrogels create an acidic environment that hinders the growth of pathogens (Yang et al., 2020).

Gelation of fresh cells can also help maintain cell viability at higher temperatures, whilst providing a vehicle for administration to the patient. Using cosmetically safe thickeners, Karoglan and colleagues resuspended freshly cultured C. acnes cells in 0.25% peptone which was solidified into a hydrogel using hydroxyethyl-cellulose 250 HX. This formulation was frozen for long-term storage but was recorded to remain stable for over 1 week at room temperature (Karoglan et al., 2019).

Using calcium chloride as an ionic crosslinker, and PVA, poly(vinyl pyrrolidone) and alginate as the polymers, R. mucosa was incorporated into a skin dressing called Hy@Rm used for the treatment of AD. As both PVA and alginate can be utilised as carbon sources by some bacteria, authors suggest that the hydrogel provides the environmental structure and nutrition required for the growth of R. mucosa, reducing competition with local commensals whilst allowing for diffusion of compounds that combat S. aureus (Liu et al., 2023a). Another gelator Pluronic F-127 was used to thicken B. subtilis NCIB 3610 cells in LB broth into a thermosensitive gel that is in a liquid form at room temperature but becomes solid when warmed to skin temperature. The Bacillus cells were shown to be viable for 24 hours in the formulation and inhibited Candida fungal growth in the mouse model (Lufton et al., 2018).

More complex preservation methods have been proposed for treating skin conditions, particularly wound healing, but have not been implemented in human clinical trials. For example, S. epidermidis cells were encapsulated in polyethyleneimine and then incorporated into fibres of carboxymethylcellulose and polyethylene oxide by electrospinning (Kurečič et al., 2018). Another similar study incorporated L. plantarum ATCC 8014 into electrospun fibres, alongside human adipose-derived mesenchymal stem cells and fibroblast cells, to promote wound healing (Shahghasempour et al., 2023).
Lyophilised LBPs

As all these methods incorporate freshly cultured cells, the loss of fitness during processing may be reduced but these systems may not be as effective at preserving the microorganisms over the long term. Traditionally, lyophilisation is the primary method for stabilising beneficial microorganisms, improving their resilience to temperature and environmental fluctuations. Many studies opt for the utilisation of lyophilised microorganisms, as freeze drying is a well-established stabilisation method for orally consumed LBPs. This process enhances the retention of viability during storage by reducing the water activity level. During lyophilisation, the cells are frozen (often within a protective solution) and then water is removed under vacuum. This reduction in cellular water activity places the microorganisms in a state of suspended animation. Upon rehydration in the environment, the bacteria become reactivated, resuming normal metabolic activities. As the result is a dry powder containing the microbes, is traditionally dispensed into a sealed vial, capsule, or sachet. This approach shields the hygroscopic powder from exposure to air, moisture, and potential contaminants (Meng et al., 2008).

As the probiotic industry has been lyophilising beneficial strains for many years, the process has been optimised and standardised for quality control. However, applying powder directly to the skin is not appropriate. Instead, they are typically reconstituted in an appropriate aqueous buffer solution before application to the patient (Myles et al., 2018; Silverberg et al., 2023). This process is easily managed when using animal models or in a clinical setting. Patients may need initial training on how to add lyophilised cells directly to an aqueous solution at home, as outlined by Casari et al. In this study, lyophilised *L. paracasei* m.biome LiveSkin88TM cells were added to a buffer directly by the patient at the time of use using a plunger device. The buffer contained hyaluronic acid and its derivatives, combining the effects of the probiotic and traditional chemical methods to improve the skin by multiple mechanisms (Casari et al., 2022). Aqueous, non-viscous solutions like these (and others previously mentioned) can be used in a spray bottle for easy, dispersed delivery of the bacteria to the skin. Otherwise, the lyophilised powder can be formulated into a more complex cream or ointment, suitable for supporting the viability of the bacteria and promoting skin healing. The LAB, *W. viridescens* UCO-SMC3 was first freeze-dried and the cells were added to an aqueous cream
containing stearyl alcohol, cetyl alcohol, sodium lauryl sulphate and propylene glycol. This cream was kept in the fridge to preserve viability, but no testing was done to quantify this (Espinoza-Monje et al., 2021).

Oil-based formulations have been trialled numerous times. Lyophilised cells can be sensitive to environmental humidity, which leads to a loss of viability. The addition of freeze-dried cells to an oil base provides a hydrophobic barrier that reduces contact between cells and damaging moisture. The lipid may also provide protective effects that reduce oxidative damage to anaerobic microorganisms. For example, when manufacturing the multi-strain LAB product YUN, cells are lyophilised and then encapsulated with an alginate polymer, before being incorporated into an oil-in-water cream (Lebeer et al., 2022). Ointments with an even lower water content may remain longer on the surface of the skin (Buhse et al., 2005), which may facilitate colonisation by the microorganisms. For these reasons, companies such as MatriSys stabilise their *S. hominis* A9 cells in an anhydrous oil carrier that supports viability for over 1 year at room temperature (Schmidt, 2020).

Lyophilised *Micrococcus luteus* Q24 was loaded into medium chain triglycerides (MCT) and thickened with silica dioxide to produce an oily serum containing 1% w/w bacteria. This safe, tolerable, serum stabilised the cells for 24 months and increased the *M. luteus* count, whilst decreasing the prevalence of pathogenic *S. aureus* on the skin. This promising trial demonstrated the successful colonisation of the skin by *M. luteus*, and future work hopes to establish the efficacy of the treatment for skin conditions (clinical trial recruiting – NCT05750381). Furthermore, the study compared the continued viability of the cells in both oil and aqueous creams, showing that the oil serum supported the preservation of *M. luteus* at room temperature for 2 years, whereas the aqueous serum was not as successful. This trial demonstrates the need for complex pharmaceutical-grade skin creams, especially formulated for the stabilisation and delivery of living microorganisms to the skin (Jain et al., 2022).

As previously mentioned, Butler et al. (with BioGaia) formulated lyophilised *L. reuteri* DSM 17938 into an oil-based product containing shea butter and canola oil for application to children affected with AD. The study monitored the viability of microorganisms at suitable temperatures throughout
and found that it remained stable. The ointment reportedly significantly improved the SCORAD index after 4 and 8 weeks of use, reduced itching, and improved sleep. However, there was no significant difference in any beneficial outcome when compared the placebo, vehicle-only control (Butler et al., 2020). This may suggest that enhanced hydration of the skin or other chemical mechanisms is mostly responsible for these bettered clinical outcomes. The preceding ex vivo skin studies had positive outcomes using this same bacterial strain lyophilised and suspended in MCT and sunflower oil. In that case, it was demonstrated that the lipids did not contribute to the antibacterial properties of the probiotic, but the effect on the skin by the lipids was not considered (Khmaladze et al., 2019).

Conclusions

As the understanding of dermatological conditions advances, it is increasingly clear that traditional pharmaceutical interventions often struggle to address the complexities of these diverse afflictions. The delicate balance of skin microbiota and its profound impact on skin health and conditions have propelled LBPs, into the spotlight as a novel approach to ameliorate various dermatological ailments. LBPs have exhibited significant promise for the treatment of key dermatological concerns, including acne and AD, and notably, wound healing. However, it is crucial to acknowledge that successful interventions heavily depend on careful strain selection. Thoughtful consideration of probiotic strains tailored to specific dermatological conditions is paramount for achieving favourable outcomes.

Moreover, the formulation of LBPs presents a set of intricate challenges. Ensuring the viability of probiotics while maintaining compatibility with the skin's unique environment remains a non-trivial task. Whilst these complexities must be addressed to fully unlock the potential of LBPs in dermatological applications, the emergence of LBPs has the potential to redefine the landscape of dermatological treatments. Future research and development efforts, guided by a deeper understanding of microbial interactions and innovative formulation techniques, hold the promise of delivering safe, effective, and personalized LBP-based solutions for a wide spectrum of dermatological conditions. As the journey continues, the collaboration between scientific inquiry and
clinical application will be instrumental in realising the full potential of LBPs as a cornerstone in dermatological care.

Conflict of Interest

SD is an employee of SGS. Quay Pharmaceuticals develop formulations of Live Biotherapeutics. AJM and RGL received research funding from SGS – Quay Pharmaceuticals and Innovate UK. AJM and CO hold patents in the area of live Biotherapeutics.
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Authors contribution statement

Conceptualisation JL, AJM, RGL, Formal analysis, JL, Funding acquisition, AJM, RGL, Supervision, AJM, RGL, Writing (original draft), JL, HS. Writing (review and editing), All authors.
Figure 1: Overview of potential mechanisms of action for LBPs on skin health, highlighting their involvement in immune modulation, pathogen inhibition, and enhancement of wound healing processes. AIPs, autoinducing peptides; AMPs, antimicrobial peptides; ROS, reactive oxygen species; TNFR2, tumour necrosis factor 2; Th2, T helper 2
Figure 2: Cross-sectional schematic of skin structure with microbial (bacteria, fungi and viruses) colonisation across the surface of the skin (stratum corneum) and deep within sebaceous glands, hair follicles and sweat glands.
Table 1: Microorganisms used in clinical trials investigating the efficacy of topically applied probiotics or LBPs for the treatment of dermatological conditions.

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