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Review

Restoring Ocular Microbiota Balance: A New Bioprinted Approach to Treating Anterior Segment Diseases

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Abstract

The ocular surface microbiota plays a fundamental role in maintaining ocular health, offering protection against pathogens, modulating immune responses, and supporting tear film stability. Dysbiosis, or disruption of this microbial balance, is increasingly recognized as a contributing factor to various ocular surface and anterior segment pathologies, including dry eye disease, blepharitis, conjunctivitis, and keratitis. This review explores the critical functions of the ocular microbiota and the consequences of its alteration in disease progression, particularly through mechanisms such as inflammation, biofilm formation, and epithelial damage. Current therapeutic approaches, including antibiotics and probiotics, face significant limitations in restoring microbial balance. A key challenge in these therapies is the delivery of beneficial bacteria in suspension, where they are rapidly cleared from the ocular surface, limiting their ability to establish a stable, protective population. To address this issue, a novel approach is proposed: the use of bioprinting technology to deliver beneficial bacteria in a solid, structured form. This bioprinted approach offers several advantages, including improved retention of bacteria on the ocular surface and a controlled release over time, increasing the likelihood of successful repopulation with beneficial microbial species. By overcoming the rapid clearance observed with conventional suspensions, this method holds the potential to restore ocular microbial balance more effectively, providing long-term therapeutic benefits for chronic ocular surface diseases. Future research will require preclinical and clinical studies to validate the safety and efficacy of this innovative approach. This bioprinted bacterial therapy represents a significant advancement in the management of ocular surface and anterior segment pathologies, offering a novel, targeted strategy for microbiota restoration.

Keywords: eye; ocular surface; microbiota; bioprinting

1. Introduction

The ocular surface microbiota (OSM) is a dynamic and diverse community of microorganisms, primarily composed of bacteria, with smaller contributions from fungi and viruses. This ecosystem plays an essential role in maintaining ocular health by interacting with the host's immune system and contributing to the stability of the tear film [1,2].

The tear film, rich in antimicrobial molecules like lysozyme, lactoferrin, and β-defensins, plays a critical role in regulating microbial populations and maintaining the balance of the microbiota [3,4]. Epithelial cells of the ocular surface further contribute by producing immunomodulatory cytokines and serving as a physical barrier against microbial invasion [3,4].

Beyond pathogen defense, the OSM plays a key role in modulating the immune response. For instance, *Corynebacterium mastitidis* stimulates the production of interleukin-17 (IL-17) by $\gamma\delta$ T cells, promoting the release of antimicrobial proteins and supporting the innate immune defense without inducing harmful inflammation [5]. This balanced interaction between the host and its microbiota is vital for preventing excessive immune reactions that could damage ocular tissues.

However, disturbances in this balance, known as dysbiosis, can favor the insurgence of ocular surface pathologies [1]. Dysbiosis can be triggered by factors like antibiotic use, environmental changes, or systemic conditions, resulting in the overgrowth of pathogenic species like *Staphylococcus aureus* or *Pseudomonas aeruginosa* [1,6]. Such disruptions impair the tear film, trigger inflammation, and exacerbate ocular conditions (Fig. 1).

Maintaining a healthy ocular microbiota is therefore crucial for eye health. Ongoing research aims to better understand these microbial interactions and develop strategies to restore balance when dysbiosis occurs [7]. This manuscript seeks to explore the critical role of the OSM in ocular health and its contribution to disease, while proposing an innovative therapeutic approach. We suggest using bioprinted beneficial bacteria, delivered in a solid, stable form, to enhance retention and colonization on the ocular surface. This method offers a promising alternative to traditional bacterial suspensions, which are quickly cleared from the eye, potentially providing a new solution for the treatment of chronic ocular surface conditions [8].

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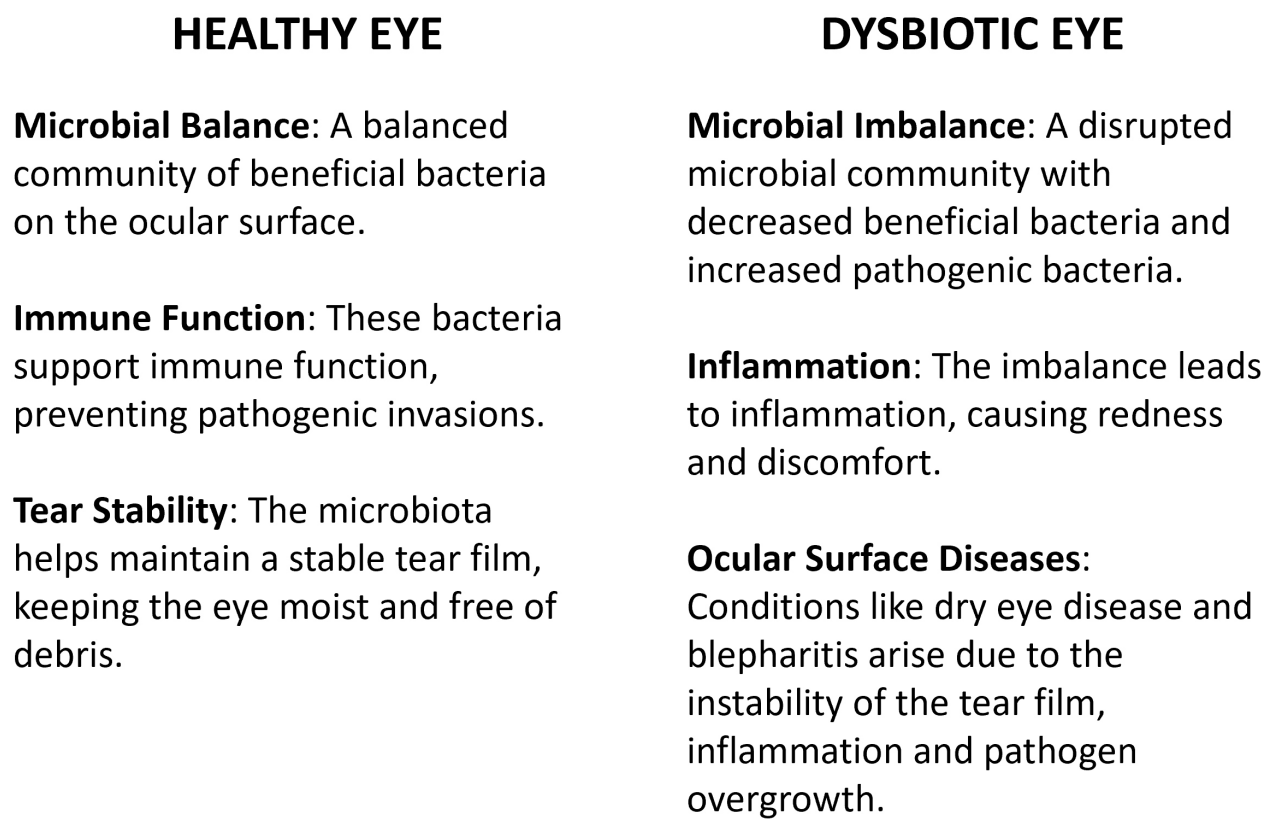

Fig. 1. Microbial balance in ocular health. Illustration of a healthy eye in which the presence of beneficial bacteria overwhelms and controls the presence of pathogenic ones, vs a dysbiotic eye, in which a microbial imbalance characterized by a decrease of beneficial bacteria favors the proliferation of potentially pathogenic microorganisms, triggering inflammation and tear film instability, thus leading to different types of ocular diseases. In this illustration 'balance' does not imply equilibrium, but a right proportion of beneficial vs. potentially pathogenic microorganisms.

2. Pathologies of the Ocular Surface and Anterior Segment

The normal ocular microbiota plays a crucial role in maintaining the delicate balance of the ocular surface. The imbalance in this microbial ecosystem, can lead to an overgrowth of pathogenic species and a reduction in microbial diversity, both of which contribute to ocular surface diseases. Dysbiosis is known to disrupt the tear film, weaken epithelial barriers, and induce chronic inflammation, all of which exacerbate ocular pathologies such as dry eye disease (DED), blepharitis, conjunctivitis, and keratitis. These alterations in the microbial community can drive inflammation, infection, and destabilization of ocular surface homeostasis.

2.1 Dry Eye Disease (DED)

Dry eye disease is one of the most common ocular surface disorders, often associated with alterations in the microbiota. Patients with DED frequently exhibit a decrease in microbial diversity, along with an overrepresentation of certain pathogenic species such as *Staphylococcus aureus* and *Corynebacterium* [9–11]. This microbial imbalance exacerbates the already unstable tear film, further promoting inflammation and discomfort. Dysbiosis may also affect the production of lipids by meibomian glands, leading to evaporative dry eye by destabilizing the tear film [12]. Recent studies suggest that reestablishing microbial balance on the ocular surface [10], or in the gut [6,13] may be a potential therapeutic target for managing DED.

2.2 Blepharitis

Blepharitis, an inflammation of the eyelids, is another ocular surface condition closely linked to microbiota dysbiosis [14]. In anterior blepharitis, also known as staphylococcal blepharitis, the eyelid margin often experiences an overgrowth of bacteria, mainly coagulase-negative staphylococci (CoNS) like *Staphylococcus epidermidis*, and to a lesser extent, *Staphylococcus aureus*. These bacteria release toxic substances into the tear film, prompting the production of proinflammatory cytokines and attracting inflammatory cells. This results in inflammation driven by both the body's immune response and the bacteria themselves. In contrast, the bacterial role in posterior blepharitis, associated with meibomian gland dysfunction, is less direct. While *Propionibacterium acnes* and *Staphylococcus epidermidis* are commonly found in these cases, they may

only contribute indirectly to symptoms. A proposed mechanism is that these bacteria release lipases that degrade the meibum, producing free fatty acids and soaps, which irritate the eye and destabilize the tear film [14]. Furthermore, Demodex mites, often found in higher numbers in individuals with blepharitis, can exacerbate microbial imbalances by disrupting the local environment [15]. The inflammatory response triggered by bacterial overgrowth results in redness, irritation, and tear film instability, characteristic symptoms of blepharitis [16].

2.3 Conjunctivitis

Conjunctivitis, or inflammation of the conjunctiva, can be of viral, bacterial, or allergic origin. Bacterial conjunctivitis is often linked to dysbiosis, with species like *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* frequently implicated [17]. In healthy individuals, these bacteria are present in small numbers, but an imbalance in the microbiota can allow them to proliferate, leading to infection and inflammation. Dysbiosis not only promotes pathogen overgrowth but also weakens the protective immune responses of the conjunctiva, making the ocular surface more susceptible to infections [18].

2.4 Keratitis

Keratitis is an inflammation of the cornea that can have bacterial, viral, fungal, or protozoan origins. In keratitis, the loss of microbial diversity has been shown to weaken the corneal immune defense, allowing pathogens to invade and cause severe infection [19]. Bacterial keratitis is particularly severe, with *Pseudomonas aeruginosa* and *Staphylococcus aureus* being the most common pathogens involved [20]. Contact lens wearers are particularly at risk, as the lens can disrupt the ocular surface microbiota, creating an environment conducive to pathogenic colonization [21,22]. Dysbiosis in keratitis often leads to aggressive inflammation, corneal damage, and, if untreated, can result in vision loss [23,24]. Therefore, restoring the balance of the ocular microbiota through probiotic therapies or by modulating the tear film environment could be proposed as a strategy to prevent recurrent infections in patients susceptible to keratitis [25]. These strategies aim to prevent pathogenic colonization and reduce inflammation, offering a promising approach for managing chronic ocular surface diseases linked to dysbiosis [26].

2.5 Uveitis

Uveitis is an inflammatory condition affecting the uvea, the middle layer of the eye. The exact etiology of uveitis is often multifactorial, involving genetic, environmental, and microbial factors. Currently, there is limited direct evidence of a causal link between ocular surface dysbiosis and uveitis, though several studies focus on the potential impact of extraocular microbiota dysbiosis on uveitis [27,28]. The mechanisms by which dysbiosis could influence uveitis involve complex interactions between microbial communities and the host immune system. Extraocular dysbiosis may lead to an overproduction of pro-inflammatory cytokines and an impaired immune response, fostering a local environment conducive to inflammation. This can result in the breakdown of the ocular surface barrier and an increased susceptibility to uveitis [4,29]. Therefore, understanding the complex role of the microbiota in the pathogenesis of uveitis could be essential for developing new therapeutic approaches. Maintaining a healthy microbial balance on the ocular surface could also be a potential strategy to prevent or mitigate uveitis. Further research is needed to elucidate the specific mechanisms and to explore targeted treatments that address microbial dysbiosis in uveitis.

2.6 Aqueous Humor and Glaucoma

The role of the ocular surface microbiota in relation to aqueous humor, trabecular meshwork, and glaucoma is an emerging area of research. The composition of the aqueous humor can be influenced by the presence of microbes on the ocular surface, potentially affecting intraocular pressure (IOP) and the risk of glaucoma. Dysbiosis or imbalance in the ocular surface microbiota can lead to inflammation and changes in the trabecular meshwork, potentially contributing to increased IOP and glaucoma. Emerging evidence suggests that the ocular surface microbiota and tear proteome may play a role in the pathogenesis of glaucoma [2,30]. Studies have found associations between specific microbial communities and the presence of glaucoma, indicating that the microbiota could influence disease progression. Overall, understanding the interactions between the ocular surface microbiota and the components of the eye could lead to new therapeutic approaches for managing glaucoma and other ocular diseases [2,30].

3. Mechanisms of Microbiota Involvement in Ocular Disease

3.1 Immune Modulation by the Ocular Microbiota

A balanced ocular microbiota supports immune tolerance, helping prevent inflammation that could compromise vision. Immune regulation on the ocular surface is maintained by antimicrobial peptides (AMPs), mucins, and secretory immunoglobulin A (sIgA), which collectively modulate microbial populations and deter pathogenic overgrowth [31,32]. Dysbiosis, characterized by a reduction in beneficial microbes and an increase in pathogenic species, can disrupt this equilibrium, prompting immune activation and chronic inflammation [33]. Pathogen-induced activation of toll-like receptors (TLRs) on ocular epithelial and immune cells leads to the release of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6), which contribute to epithelial damage and inflammation. This persis-

tent activation can engage both innate and adaptive immune cells, perpetuating a cycle of chronic inflammation [29].

3.2 Gut-Eye Axis and Systemic Immune Crosstalk

Beside the resident ocular surface microbiota, there is a well-known gut-eye axis supporting a bidirectional relationship between the gastrointestinal microbiota and ocular immunity. Research suggests that immune activation resulting from gut dysbiosis can influence ocular immune responses, exacerbating or even triggering ocular diseases [34]. For example, gut-derived T-helper cells and pro-inflammatory cytokines can migrate to the ocular surface, promoting inflammation [35]. This crosstalk is particularly relevant in autoimmune uveitis, where gut dysbiosis has been shown to intensify ocular inflammation, suggesting that modulating the gut microbiota might offer a promising approach for achieving systemic and ocular immune balance [36].

3.3 Microbial Metabolites in Inflammatory Pathways

Microbial metabolites influence host immune responses and ocular health. Short-chain fatty acids (SCFAs) produced by commensal bacteria exert anti-inflammatory effects by modulating immune cells. A decrease in SCFA-producing bacteria due to dysbiosis reduces these protective effects, leaving the ocular surface susceptible to inflammation [4,6,37]. In contrast, dysbiotic microbiota may increase harmful metabolites, such as lipopolysaccharides (LPS) from Gram-negative bacteria. LPS activates TLRs on ocular epithelial cells and immune cells, triggering pro-inflammatory cytokine production and increasing the risk of inflammatory eye diseases like uveitis [29].

3.4 Pathogen Colonization and Biofilm Formation

Pathogenic bacteria, including *Staphylococcus aureus* and *Pseudomonas species*, can establish persistent colonies on the ocular surface through biofilm formation, a significant factor in chronic ocular infections. Biofilms are structured microbial communities encased in an extracellular matrix that enhances bacterial adhesion to the ocular surface, making bacteria up to 1000 times more resistant to antibiotics and immune responses compared to free-floating (planktonic) bacteria [38,39]. These biofilms shield bacteria from immune clearance, contributing to conditions like blepharitis and bacterial keratitis. The biofilm matrix alters the microenvironment of the ocular surface, including pH, oxygen levels, and nutrient availability, which weakens the host's defenses and fosters recurrent infections [29,39]. This persistent bacterial presence complicates treatment, as biofilms prevent effective antibiotic penetration. Alternative treatments, such as lactoferrin or DNase enzymes to degrade biofilm structures, are under investigation, but more research is needed to confirm their safety and efficacy for ocular use [40,41].

On the other hand, if it were possible to produce a beneficial biofilm with non-pathogenic, symbiotic bacteria, this could potentially shield the ocular surface from infections. This approach leverages the competitive interactions that naturally occur between bacterial communities. In fact, a beneficial biofilm could outcompete pathogenic bacteria for resources and space on the ocular surface, making it harder for harmful bacteria to establish themselves. A stable, beneficial biofilm could provide a physical barrier that limits the adhesion of pathogens to the ocular surface, blocking entry points for pathogens. Moreover, non-pathogenic bacteria within a beneficial biofilm may help modulate local immune responses, reducing excessive inflammation that could otherwise damage the tissue and make it more vulnerable to infection. Finally, beneficial bacteria might also produce antimicrobial peptides or other molecules that inhibit pathogen growth directly. In short, fostering a beneficial biofilm could indeed offer a protective shield, reducing the risk of infections and promoting ocular health. This concept aligns well with the idea of using a bioprinted device to deliver beneficial bacteria, as it could help establish this protective layer precisely where needed on the ocular surface.

4. Potential Therapeutic Approaches Targeting the Ocular Surface Microbiota

Currently, the management of ocular surface diseases more often involves the use of antibiotics, anti-inflammatory drugs, and probiotics. These treatments aim to control infection, reduce inflammation, and restore microbial balance. Antibiotics are commonly used to treat bacterial infections of the ocular surface. They work by eliminating pathogenic bacteria, thus resolving the infection and preventing its spread. Examples include fluoroquinolones and macrolides, which are effective against a wide range of ocular pathogens [6]. Anti-inflammatory drugs, such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs), are employed to reduce inflammation and alleviate symptoms associated with ocular surface diseases like uveitis and blepharitis. These drugs help to control immune responses and minimize tissue damage [42]. Probiotics are beneficial microorganisms that are usually administered orally, and may restore the natural balance of the ocular surface microbiota through the gut-eye axis. Probiotic treatments have shown promise in managing conditions like dry eye disease by enhancing microbial diversity and stabilizing the tear film [34,36].

Despite their benefits, current therapies for ocular surface diseases come with significant limitations. The overuse of antibiotics can lead to the development of antibiotic-resistant bacteria, which poses a challenge for treatment. Resistant strains can persist on the ocular surface, making infections harder to treat and increasing the risk of recurrence [43]. Antibiotics and anti-inflammatory drugs can disrupt the balance of the ocular microbiota by

eliminating not only pathogenic bacteria but also beneficial ones. This disruption can lead to dysbiosis, which may exacerbate symptoms and make the ocular surface more susceptible to future infections [34,35]. Due to the limitations of existing therapies, there is a risk of recurring infections. The inability to completely eradicate pathogens or restore microbial balance can result in chronic or recurrent ocular surface diseases [4].

Therefore, new therapeutic approaches are being explored to address the limitations of current treatments and improve outcomes for patients with ocular surface diseases. Fecal microbiota transplantation (FMT) has been used successfully to restore gut microbiota in patients with recurrent Clostridium difficile infections. Inspired by this approach, researchers are investigating ways to apply similar principles to the ocular surface. This involves transplanting healthy ocular microbiota to restore balance and prevent dysbiosis-related diseases [34,44]. Topical probiotics are being studied as a way to directly apply beneficial microorganisms to the ocular surface. These probiotics aim to enhance microbial diversity, stabilize the tear film, and reduce inflammation. Early research indicates that this approach could be effective in managing conditions like dry eye disease and bacterial keratitis [6,35]. More specifically, the potential of probiotics and postbiotics has been explored for the management of ocular surface diseases, offering innovative approaches to reduce inflammation and restore microbial balance. A study by Iovieno *et al*. (2008) [45] demonstrated the effectiveness of *Lactobacillus acidophilus* eye drops in treating vernal keratoconjunctivitis (VKC). Over a one-month period, patients showed notable improvements in both clinical signs and symptoms, with a concurrent reduction in inflammatory markers such as Intercellular Adhesion Molecule-1 (ICAM-1) and TLR-4. These findings suggest that probiotics can modulate ocular inflammation and alleviate symptoms in VKC, although the study's open-label design and small sample size limit its generalizability, emphasizing the need for larger, double-blind controlled trials. Building on the role of microbiota in ocular health, Layús *et al*. (2022) [46] developed an ophthalmic formulation containing a postbiotic derived from *Lactiplantibacillus plantarum* CRL 759. This formulation demonstrated significant anti-inflammatory effects *in vitro*, reducing pro-inflammatory mediators in lipopolysaccharide-stimulated murine macrophages. While these results indicate the potential for postbiotics to address inflammation in ocular surface diseases, the study was limited to *in vitro* models, requiring further *in vivo* and clinical research to assess its safety and efficacy in humans. Expanding on these advancements, Heydari *et al*. (2024) [47] investigated the use of *Latilactobacillus sakei* in treating DED through both topical and systemic formulations. Their randomized, placebo-controlled trial revealed that topical application significantly improved clinical signs and symptoms of DED while suppressing ocular surface inflammatory markers, with systemic administration showing less pronounced effects. However, the study's short-term evaluation period and specific patient cohort highlight the need for further research to confirm long-term safety and efficacy across broader populations. Collectively, these studies demonstrate the promise of probiotics and postbiotics in treating ocular surface diseases by modulating inflammation and promoting microbial balance. Nonetheless, limitations such as small sample sizes, reliance on preclinical models, and a lack of long-term data underscore the need for further standardization and clinical validation to establish these therapies as viable alternatives to conventional treatments.

In conclusion, while current treatments for ocular surface diseases have been effective to some extent, they come with notable limitations, including the risk of antibiotic resistance and disruption of the beneficial microbiota. Emerging approaches, such as FMT-inspired concepts and topical probiotics, offer promising alternatives that could enhance the management of ocular surface diseases by restoring microbial balance and reducing inflammation. Continued research and clinical trials are necessary to confirm the safety and efficacy of these innovative therapies.

5. Proposal of a New Medical Device: Bioprinted Beneficial Bacteria

The present proposal, by its own nature, is hypothetical and is based on indirect evidence. To the best of my knowledge, there are currently no scientific reports on bacterial bioprinted devices designed to deliver beneficial bacteria to the ocular surface. However, the technology appears to be sufficiently advanced and requires only thorough testing in appropriate preclinical and clinical models. This makes the present report a novel and promising opportunity for the development of such a device.

Bioprinting technology, an innovative branch of 3D printing, enables the precise placement of living cells and biomaterials to create tissue-like structures. This technology has rapidly evolved and holds significant potential in regenerative medicine and microbial therapy. By utilizing bioprinting, researchers can create complex, organized structures that closely mimic natural biological tissues [48].

Bacterial bioprinting, an emerging frontier at the intersection of 3D printing technology and microbiology, holds a big promise for revolutionizing materials engineering. This innovative approach, as demonstrated by Schaffner *et al*. [49], involves embedding bacteria in biocompatible and functionalized 3D printing inks, enabling the creation of "living materials" with diverse functionalities. This intersection of additive manufacturing and bacterial diversity presents unprecedented opportunities for biotechnological and biomedical applications. In a seminal study [50], bacterial species were ingeniously mixed with bioinks

to produce complex functional materials using 3D printing. This opened avenues for applications such as bioremediation, toxin detection sensors, oil spill filters, and advanced wound dressings. The concept of 3D-printed mini-biofactories, elucidated by Kyle [50], representing a potential paradigm shift in biotechnology. Further advancements delve into the realm of bacterial biofilms, intricate three-dimensional networks [51]. These biofilms, entangled in self-generated extracellular matrices, exhibit remarkable resilience to adverse conditions.

The 3D printing platform developed by Balasubramanian and colleagues [51] introduces genetically engineered *Escherichia coli* biofilms, thus extending the applications to bioleaching, bioremediation, and materials production. Along this line, another recent work [52] focused on the printing of a series of 3D gelatin-based hydrogels immobilized with fermentation bacteria that can secrete hyaluronic acid (HA), a very useful natural polysaccharide in the fields of biomedicine and tissue engineering. Moreover, as antimicrobial resistance becomes a global concern, another work [53] provided a groundbreaking approach by 3D bioprinting clinically relevant bacterial species. The resulting biofilms, exhibiting resistance to antimicrobials, provide a clinically relevant testing model surpassing traditional 2D cultures. This brings forth the potential for advanced antimicrobial testing methodologies. In order to elevate the bacterial bioprinting technology, a new "microbial ink" for 3D printing of living materials has been introduced [54]. This innovative ink, produced entirely from genetically engineered microbial cells, facilitates self-assembly and hierarchical organization. The resulting 3D-printed living materials can sequester toxic moieties, release biologics, and regulate cell growth. In the realm of sustainable construction materials, the incorporation of cyanobacteria into 3D-printed biomaterial inks has been proposed [55]. This introduced the concept of living building materials (LBM) that can produce calcium carbonate ($CaCO_3$) as a bio-cement. Such materials have the potential for applications in environmentally friendly construction, including art restoration and marine reef regeneration. All in all, bacterial bioprinting emerges as a transformative technology, weaving together the principles of microbiology and materials science. The cited studies collectively underscore the versatility of this approach, ranging from biotechnological applications and antimicrobial testing to the creation of sustainable building materials. As researchers continue to unravel its potential, bacterial bioprinting stands poised to reshape the landscape of materials engineering and open doors to unprecedented applications across diverse domains.

These procedures offer a promising avenue also for the integration of local microbiota, by delivering beneficial bacteria to specific anatomical sites, such as the ocular surface, where they can exert therapeutic effects. Indeed, there is a good rationale for using beneficial bacteria on the ocular surface in a solid form. Traditional topical formulations, such as eye drops or ointments, often face challenges in delivering therapeutical compounds on the ocular surface. In fact, these formulations are typically cleared rapidly by the tear film and the blinking action, limiting their efficacy [56]. Targeting beneficial bacteria in a solid form, using bioprinting technology, may offer several advantages. The structured form can adhere better and stay longer onto the ocular surface, as is the case for drug-releasing dissolvable contact lenses [57], thus potentially allowing for a controlled release of bacteria over time. This sustained presence increases the likelihood of successful colonization and therapeutic impact. Therefore, the bioprinting technology will enable the creation of a structured delivery mechanism that can be applied to the ocular surface. This bioprinted form can encapsulate beneficial bacteria within a biocompatible matrix, allowing for controlled release and stability. The structured form ensures that the bacteria remain in contact with the ocular surface for an extended period, overcoming the rapid clearance seen with conventional suspensions. This prolonged presence enhances the chances of effective colonization and sustained therapeutic benefits. One of the primary benefits of this bioprinted approach is the restoration of microbial balance on the ocular surface. Dysbiosis, or microbial imbalance, is implicated in various ocular surface diseases. By delivering beneficial bacteria in a controlled manner, this therapy can re-establish a healthy microbial community, reducing the risk of infection and inflammation. The sustained release of beneficial bacteria will offer long-term protection against pathogenic microorganisms. These beneficial strains can outcompete harmful bacteria, produce antimicrobial compounds, and stimulate the host's immune response. This protective effect helps prevent recurrent infections and maintains overall ocular health. Chronic ocular surface conditions, such as dry eye disease and blepharitis, often involve inflammation and epithelial damage. The bioprinted beneficial bacteria could promote healing by reducing inflammation, supporting epithelial regeneration, and enhancing the stability of the tear film. Indirect evidence for this activity may come from the review article by Petrillo *et al*. [4], highlighting how non-pathogenic bacteria can support the colonization of a healthy microbiota, which in turn can modulate immune responses and promote tissue repair. This comprehensive approach addresses the underlying causes of these pathologic ocular conditions, providing long-term relief and improved quality of life for patients.

Obviously, the selection of appropriate bacterial strains is crucial for the success of this bioprinted therapy. The definition of the abundance and composition of the OSM in healthy subjects is highly variable in different studies and strictly dependent on the methods used, which include cultured-dependent and the more recent culture-independent methods [58]. The OSM identified in healthy subjects by the cultured-dependent techniques appears almost entirely composed by bacteria, mainly including the

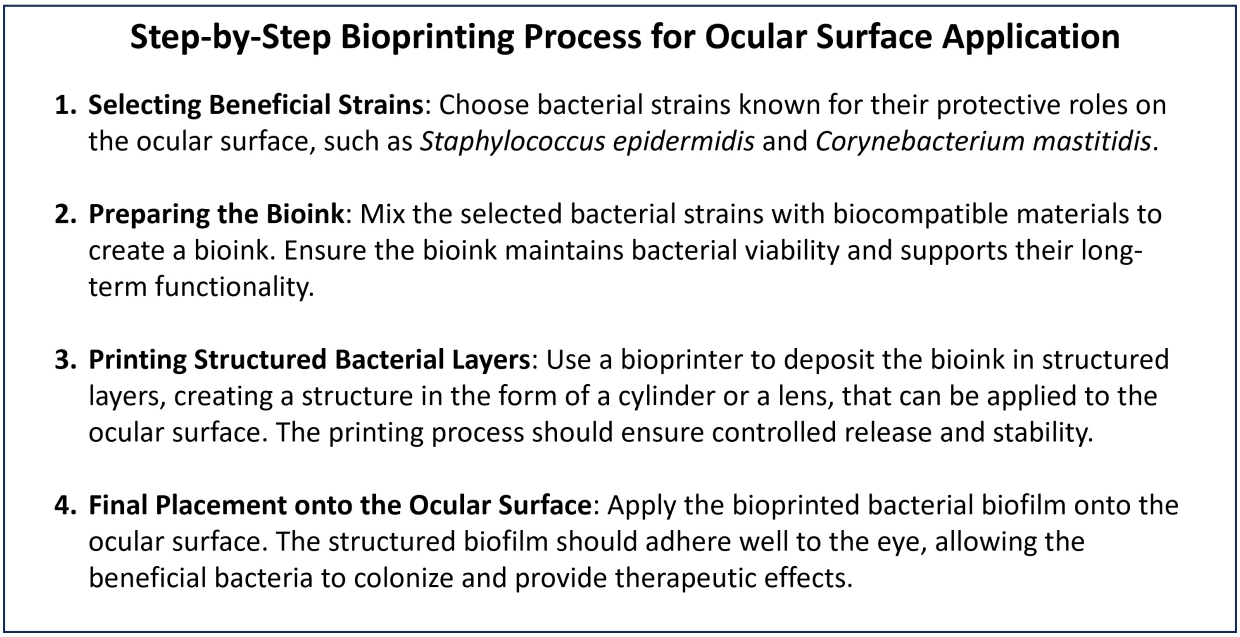

Fig. 2. The diagram illustrates the steps for the production of implantable devices releasing beneficial bacteria on the ocular surface.

genera coagulase-negative Staphylococci, *Staphylococcus aureus*, *Propionibacterium*, *Corynebacterium* and *Streptococcus*; other microbes isolated from the OS include *Diphtheroid* bacteria, *Micrococcus*, *Escherichia*, *Enterococcus*, *Lactobacillus*, *Bacillus*, *Hemophilus*, *Neisseria*, *Pseudomonas* and fungi. The most common OS bacteria are Gram-positive coagulase-negative Staphylococci, which are present in 20–80% of the conjunctival swabs and in the 30–100% of the lid swabs. No difference between fellow eyes were found in the OSM using the traditional culture-dependent techniques [59–61].

To our purpose, possible bacterial strains to include in a probiotic for the ocular surface could belong to the following species:

• Corynebacterium: This genus is the most common on the ocular surface and is thought to play a role in maintaining the health of the tear film. *Corynebacterium mastitidis* could play a protective role by stimulating the immune system to produce antimicrobial factors, thereby preventing infection.

• Propionibacterium: This genus is also found in healthy eyes and is thought to help to break down lipids in the tear film.

• Staphylococcus: This genus is found in both healthy and diseased eyes and can contribute to dry eye disease if it overgrows. *Staphylococcus epidermidis* has a recognized role in protecting the ocular surface by producing antimicrobial peptides and competing with pathogenic bacteria.

• Pseudomonas: This genus is not typically found in healthy eyes, but it can overgrow in people with blepharitis or contact lens-related infection.

These strains are chosen for their ability to enhance ocular health, provide protection against pathogens, and support the stability of the tear film.

Therefore, based on the aforementioned premises, where the significance of ocular surface microbiota for eye health has been demonstrated, and the feasibility of bioprinting living microorganisms has also been established, we propose the development of implantable devices consisting of bioprinted bacteria. The selection of bacteria for these devices will be based on their demonstrated benefits for ocular surface and eye health. These implantable devices could take the form of a contact lens or any other shape suitable for application to the ocular surface or insertion into the inferior conjunctival fornix.

The implantable device will be molded onto a biodegradable and resorbable support, which could be either self-generated by the microorganisms, or exogenously provided in the form of, e.g., hyaluronic acid, carboxymethylcellulose, hydroxypropyl methylcellulose, polyethylene glycol, polylactic acid, etc., (Fig. 2).

Moreover, the bacteria used for this purpose could also be derived from genetic engineering manipulations, so that they could produce biomolecules useful to eye health, such as for instance hyaluronic acid or proteins deficient on the ocular surface.

In this approach, the implantable device would gradually dissolve, releasing living bacteria onto the ocular surface. This allows the bacteria to integrate into the local microbiota, potentially shifting its equilibrium towards a more beneficial state. We anticipate that this method of administering living bacteria to the ocular surface will be more effective than using living bacteria as eye drops. Eye drops

are immediately diluted in tear fluid and are quickly eliminated through drainage due to the high dynamic turnover of tears.

To maintain a stable composition of the beneficial ocular surface microbiota, it is likely that periodic applications of fresh implantable devices will be necessary. The frequency of these applications would depend on the subjective stability of the intervention.

6. Future Directions and Challenges

The bioprinting and delivery of beneficial bacteria to the ocular surface present unique challenges, primarily related to ensuring the viability and functionality of printed bacteria. To achieve successful colonization and therapeutic efficacy, it is crucial to maintain bacterial cells in a viable state post-bioprinting, especially given the mechanical and thermal stresses often involved in these processes. Furthermore, bioprinting must support the long-term functionality of these bacteria, ensuring that they can effectively integrate into the ocular surface microbiota and maintain their protective functions over time. A stable, controlled release mechanism is essential to avoid rapid clearance by the tear film or blinking, which would diminish the therapeutic potential of this intervention.

Another significant hurdle is navigating the regulatory landscape. Since bioprinted bacteria represent a novel therapeutic approach, regulatory agencies may require extensive testing and validation to confirm their safety and efficacy. The introduction of live, bioprinted bacteria also raises patient safety concerns, such as potential immune reactions, infection risk, and unintended effects on ocular health. Addressing these challenges will be critical for advancing bioprinted bacterial therapies from the laboratory to clinical practice [62].

To bring bioprinted bacterial therapies to clinical use, it will be essential to conduct rigorous preclinical and clinical testing to validate their safety, efficacy, and stability. Initial preclinical studies should investigate factors like optimal strain selection, release kinetics, and integration into the existing ocular microbiota. These studies will help to refine the bioprinting approach and delivery methods, ensuring that beneficial bacteria remain viable and effective on the ocular surface. Clinical trials will be necessary to confirm the therapeutic benefits and assess potential side effects in humans. One critical area of investigation will be the long-term effects of introducing bioprinted bacteria to the ocular surface ecosystem. Since the ocular microbiota plays a delicate role in maintaining eye health, introducing new bacterial populations, even beneficial ones, may alter this balance. It will be important to monitor for unintended impacts on microbial diversity, immune response, and potential for dysbiosis or infection over time. Evaluating the durability of these changes will help to establish protocols for periodic re-application of bioprinted bacterial devices if needed, as well as to assess how frequently treatments should be administered for optimal therapeutic outcomes [63].

In addition to bioprinting, several alternative delivery methods could be explored for the administration of beneficial bacteria to the ocular surface, each with unique strengths and limitations. Nanoparticle-based carriers, for example, offer a highly customizable platform for delivering therapeutics, as they can be engineered to encapsulate and protect bacteria from the tear film's rapid clearance mechanisms [64]. Nanoparticles, especially those made from biocompatible materials like chitosan or lipids, can improve adhesion to the ocular surface and provide sustained release, increasing the bacteria's chances of successful colonization. Additionally, nanoparticles can enhance stability and reduce degradation, an advantage in environments as exposed as the eye.

Bioadhesive gels represent another promising method, as they can form a thin, adhesive layer on the ocular surface that retains bacteria longer than standard eye drops. Hydrogels, for instance, can be tailored to respond to specific environmental triggers, such as pH or temperature, which makes them capable of providing on-demand release of encapsulated bacteria. Such gels also mimic the natural tear film, making them suitable for extended use and minimizing irritation [65].

While these methods are advantageous, bioprinting uniquely allows for a structured, solid-state delivery of bacteria that can be precisely positioned on the ocular surface in specific patterns or concentrations. Unlike nanoparticles or gels, bioprinting enables a high level of control over spatial arrangement, which may enhance therapeutic efficacy by optimizing bacterial distribution and interaction with ocular tissues. This structured format also allows for controlled layering and integration of multiple bacterial strains within a single device, which is difficult to achieve with other methods. Thus, while nanoparticle carriers and bioadhesive gels offer supportive benefits, bioprinting remains particularly well-suited for targeted and sustained ocular applications where spatial organization and long-term stability are paramount. Table 1 highlights how bioprinting offers unique advantages in spatial control and sustained delivery, making it particularly suitable for applications requiring long-term microbiota restoration. However, nanoparticles and bioadhesive gels can complement bioprinting in cases where shorter retention times or simpler formulations are adequate.

Table 2 (Ref. [49–56]) provides a comprehensive overview of the fundamental components involved in bacterial bioprinting for medical applications. The information presented includes critical insights into bioprinting technologies and strategies for bacterial encapsulation. The accompanying references offer a detailed connection to relevant research studies, highlighting advancements in the field and the innovative methodologies employed in these areas.

Table 1. Comparison of bioprinting, nanoparticle-based carriers, and bioadhesive gels for delivering beneficial bacteria to the ocular surface.

Delivery method	Advantages	Limitations	Suitability for ocular application
Bioprinting	- Allows precise spatial organization and layering of multiple strains. - Structured solid-state form improves adherence and retention. - Controlled release potential through customizable bioinks and layering.	- Complex manufacturing process with potential high costs. - Requires optimization to maintain bacterial viability during printing.	Well-suited for applications needing sustained and targeted delivery to support long-term microbiota restoration.
Nanoparticle-based carriers	- Encapsulates bacteria, protecting them from rapid clearance and degradation. - Can be engineered for sustained release of bacteria. - Flexibility in size, material composition, and functionalization.	- Risk of incomplete adherence due to tear film clearance. - Requires thorough testing for biocompatibility and potential immune reactions.	Effective for short-term retention and protection against clearance but may not ensure the prolonged colonization needed for microbiota therapy.
Bioadhesive gels	- Adheres well to ocular surface, extending retention time. - Environmental triggers (pH, temperature) can control release. - Mimics natural tear film, minimizing irritation.	- May have limited spatial organization for specific bacterial strains. - Potential challenges in controlling release duration for stable colonization.	Useful for improving bacterial retention on the ocular surface; best for applications needing moderate retention and compatibility with natural tear dynamics.

Table 2. Key elements of bacterial bioprinting for medical applications.

Aspect	Details	References
Bioprinting process	Layer-by-layer deposition of bacterial bioinks using extrusion-based or inkjet bioprinting technologies to create structured 3D scaffolds.	Schaffner *et al.*, 2017 [49]; Kyle 2018 [50]
	Incorporation of genetically engineered bacteria into bioinks for controlled therapeutic release and functionality.	Duraj-Thatte *et al.*, 2021 [54]
Bacterial strain fixation	Encapsulation of *Lactobacillus* spp, *Corynebacterium mastitidis*, or *Escherichia coli* in biocompatible hydrogels to enhance viability and therapeutic properties.	Balasubramanian *et al.*, 2019 [51]; Cui *et al.*, 2022 [52]
	Immobilization of bacteria in gelatin-based or alginate bioinks, ensuring structural integrity and controlled biodegradability.	Reinhardt *et al.*, 2023 [55]
Properties of biodegradable materials	Use of gelatin, alginate, hyaluronic acid, or carboxymethylcellulose for scaffold creation, ensuring biocompatibility, controlled degradation, and stability.	Cui *et al.*, 2022 [52]; Rupenthal & Agarwal, 2024 [56]
	Biodegradability tailored to ocular applications, allowing gradual dissolution and therapeutic release of bacteria on the ocular surface.	Ning *et al.*, 2019 [53]; Balasubramanian *et al.*, 2019 [51]

References report to research on bioprinting technologies, bacterial encapsulation strategies, and biodegradable material properties suitable for ocular use.

7. Conclusions

The ocular surface microbiota plays a critical role in maintaining ocular health, acting as a first line of defense against pathogens, supporting immune balance, and preserving the stability of the tear film. When this delicate microbial balance is disrupted (dysbiosis) an array of ocular surface and anterior segment pathologies can arise, from dry eye disease to conjunctivitis and keratitis. These conditions are often challenging to treat due to the persistence of dysbiosis, which can weaken the ocular defense mechanisms and foster inflammation and infection. The exploration of bioprinted beneficial bacteria presents a promising new frontier for restoring microbial balance on the ocular surface. Unlike conventional treatments, which are often quickly cleared or fail to establish stable microbial populations, a bioprinted bacterial device offers a unique, solid-state delivery system that may adhere more effectively, allowing for a controlled release of beneficial bacteria over time. This approach holds the potential not only to address existing dysbiosis but also to support long-term ocular surface health by creating a stable, protective microbial environment. To realize the full potential of this approach, further research is essential. There is a pressing need for innovative therapies that directly target microbiota restoration, moving beyond traditional antibiotics and short-term treatments. The proposed bioprinted device represents an initial step toward more sustainable, personalized treatments that can adapt to the unique microbial needs of each patient. As we continue to advance in understanding the interactions within the ocular microbiota, this technology may pave the way for a new era in managing chronic ocular surface diseases, reducing the recurrence of infections, and ultimately improving patient outcomes.

Author Contributions

DR, the only author of this review, was responsible for the conception of ideas presented, writing, and the entire preparation of this manuscript.

Ethics Approval and Consent to Participate

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Conflict of Interest

The author declares no conflict of interest. Dario Rusciano is serving as one of the Editorial Board members and Guest Editors of this journal. He had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article I delegated to Sarah D. Atkinson.

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