Review Article

Microbial Biofilms as a Risk Factor for *Acantahmoeba* keratitis

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Abstract

Members of the protozoan genus *Acanthamoeba* are free-living amoebas and the primary causative agent of amoebic keratitis and granulomatous encephalitis. Amoebic keratitis mainly occurs in individuals who wear soft contact lenses following trauma and exposure to contaminated sources. As the survival, growth, and multiplication of this protozoan increases in biofilms, which is the microbial accumulation, several cases of amoebic keratitis have been reported as co-infections with bacteria, especially *Pseudomonas aureginosa*. *Pseudomonas* improves the binding of this protozoan to soft lens surfaces by its special surface enzymes, resulting in *Acanthamoeba* keratitis and sometimes mixed infection. Biofilm accumulates microorganisms, including bacteria and fungi, on living and non-living surfaces. The firm attachment of these microbial accumulations to the surface can cause various diseases and resistance to treatment. The critical point is that bacteria have less destructive effects in the plantonik state, and when the specific signals of these microbiome cause the recall of other microorganisms and their interaction, they lead to problems. Unfortunately, the ocular defense barrier, which includes the eyelids, lacrimal glands, and tears, cannot kill microbial agents when microorganisms accumulate and adhere firmly to the lens surfaces. Due to the great importance of AK, especially in medical contact lens users, biofilm formation is an important issue that has been considered in recent years. Biofilm formation-induced drug resistance, which is caused by the disproportionate density of microorganisms on the inner and outer surfaces of the biofilm. There are several ways to prevent the formation of biofilms on the surfaces of contact lenses, including the use of antimicrobial peptides and various antibiotics and coating them on the inner surface of contact lenses to slow-release of antibiotics and prevent biofilm formation. In the present review article, we aimed to introduce biofilms as one of the important risk factors for developing *Acanthamoeba* keratitis and prevention ways of biofilms formation.

Keywords: *Acanthamoeba*, Keratitis, Biofilm

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Introduction

To know what a biofilm is, we first need to define planktonic and biofilm. Planktonic is when microorganisms accumulate as individual cells on the surface or inside the culture medium, and their accumulation causes the formation of biofilms[1]. In addition to bacteria, protozoa such as *Acanthamoeba* and *Vannella* spp. are also may present in biofilms[2,3].

In general, the presence of microorganisms on living

or non-living surfaces, the formation of extracellular matrix between them, and their connection to each other lead to biofilm formation[1].

Biofilms were first discovered in the microbial profiles of deep-ocean, and it was revealed that the history of the biofilm-forming ability back to 3 billion years ago. Later, in the seventeenth century, Antoine van Leeuwen-Hooke discovered many microorganisms in dental plaque that had formed biofilm while working on his sample using a microscope[4].

Protozoan genus *Acanthamoeba* is free-living that exists in various places such as soil and water, including domestic water. Also, it has been isolated from clinical samples[5, 6]. The genus can cause *Acanthamoeba* keratitis and granulomatous encephalitis, which are important diseases[7-11]. *Acanthamoeba* keratitis is a very severe irritating infection of the cornea that results in pain, diplopia, redness, and tearing, and the main symptom is infiltration, which can lead to vision loss if left untreated[12]. The risk factors of this disease are corneal trauma, contact lenses worn all over the night, exposure to contaminated waters, smoking, poor hygiene of hands, and using urban contaminated water to wash the lens[13]. Also, individuals who misuse contact lenses and cause irritations and damage to their corneal epithelium are at risk of the disease; of course, there is a discussion[12]. In the present review article, we aimed to introduce biofilms as one of the critical risk factors for developing *Acanthamoeba* keratitis and prevention ways of biofilms formation.

Methods

This study will review information about the effects of biofilms caused amoebic keratitis. The keywords searched included biofilms, keratitis, Acanthamoeba, and contact lenses in this review. We searched English reported and published articles in local and international journals over 2015-2020 using different databases, including ISI Web of Science, PubMed, and Google Scholar. Then, the related articles were reviewed.

Many articles have been published during this time, but we tried to choose and review articles that introduced biofilms and microbial colonies that caused amoebic keratitis and did not mention articles unrelated to keratitis. In total, 10 full-text articles were selected and reviewed. It should be noted that articles on other problems and diseases of biofilms have been removed from this study. Presenting this review and similar articles may help to plan future studies.

Discussion

Stages of biofilm formation: In the first stage, a reversible connection is made between planktonic cells on the surface; in the second stage, the connection becomes irreversible and strong, and in the next stage, microcolonies will be formed. Then we will see the maturation of the biofilm, and eventually, the cells will separate individually in the environment[4].

The biofilm microorganisms produce an extracellular matrix (ECM) that contains polysaccharides, proteins, nucleic acids, and lipids. Biofilms may be made up of one or more species that show different stages of intracellular differentiation and exchange of metabolites [14]. An essential issue with biofilms is the unequal distribution of nutrients and oxygen at the biofilm's surface and center; this increases microorganisms' resistance to microbial and antimicrobial substances, environmental stress, and host immune system. So that the surface microorganisms of biofilms have easier access to oxygen and nutrients and are in their active form, it causes their easily eliminated, but those in the center of the biofilm can show more resistance to harsh conditions[14].

Another interesting fact about microorganisms is quorum sensing; this means that when the concentration of the environment and the number of microorganisms increase and come out of the planktonic conditions and become biofilms, they can exchange information among themselves and activate an intracellular signal in them that can perform many metabolic activities within these biofilm conditions[15].

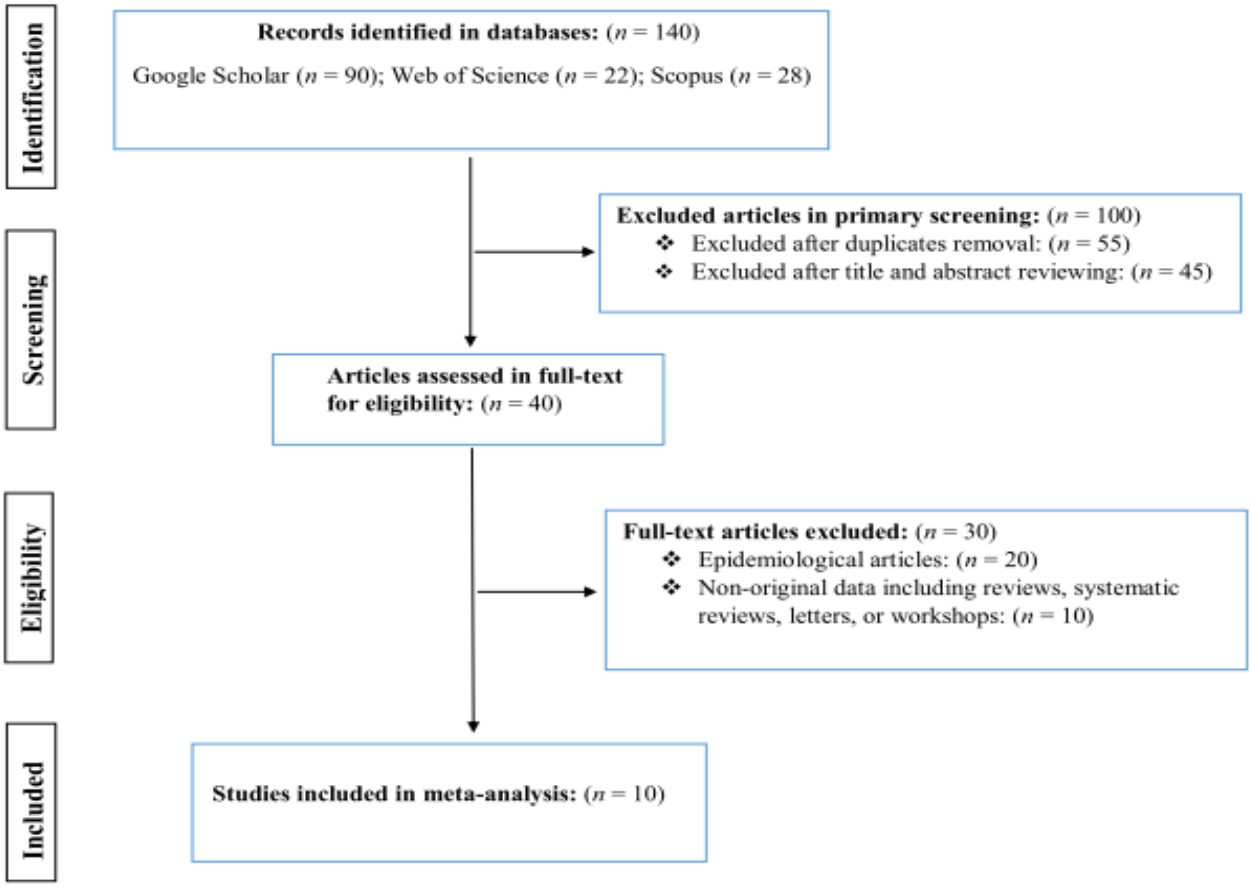

Figure 1. Diagram of included and excluded articles.

Contact lenses types: Contact lenses are classified into hard and soft based on the materials used[16]. Soft contact lenses are made of silicone or hydrogel material that allows oxygen to pass into the lens material, and the cornea's surface, and these lenses are more hydrophobic than other lenses. Hard lenses are made of polymethacrylate material with no water in them, and they are more resistant, so hard lens wearers sometimes feel discomfort in their eyes. However, microorganisms can attach to both types of lenses[4, 16, 17].

Hydrophobicity is a critical condition for the attachment of microorganisms to contact lenses. Thus, in hydrophobic surfaces, the number of attached microorganisms is more and also their connection in this type of surface is stronger than hydrophilic surfaces[12].

Contact lenses are one of the most important primary sources of infection that can contaminate the lenses and their solution. Studies have suggested that lens cases of 80% of asymptomatic contact lens users were contaminated[18].

Binding process of microorganisms to the surface: The surface binding process has two stages; including The first stage of bonding is reversible, unstable, and easily separated because the van der Waals forces present between the microorganisms are very weak and unstable. We see a more irreversible and more robust connection in the second stage. In this case, the Brownian motion, common among microorganisms, will no longer exist and will not be easily separated. At this stage, biofilm is formed as one of the most important and influential forms of life on Earth[15].

Microbial biofilms: There are various microorganisms inside the biofilm, and there are many bacteria among these microorganisms. Among these microorganisms, protozoan Acanthamoeba, which can feed on germs and bacteria inside the biofilm and causes Acanthamoeba keratitis[12]. Also, biofilms can protect Acanthamoeba against harsh environmental

conditions and thus increase its pathogenicity[19]. There are various human infections associated with biofilms, such as endocarditis, middle-ear infection, and lung infections, also microorganisms may accumulate in medical devices and implants. For example, coronary artery stents, urinary catheters, joint fittings, and most importantly, the contact lenses and intraocular lenses, the subject of our discussion, can be the site of microorganisms accumulation. Also, about eye infections that we are talking about, infectious endophthalmitis, lacrimal system infections, and keratitis are biofilm-associated, and keratitis is contact lenses-related[4].

Keratitis and biofilms: There are different types of keratitis, in which contact lenses of patients were clean and germ-free initially, but after being placed in a contaminated lens case, microorganisms such as Acanthamoeba settled on the inner surface of the lens and formed a biofilm20. Therefore, reusing this lens has led to fungal, bacterial, viral, and Acanthamoebic keratitis. It is worth mentioning that the more significant presence of Acanthamoeba in poor contact lenses is due to the presence of more bacteria[20].

Some infections of the cornea are lens-related[21]. Various microorganisms such as Mycobacterium spp., Escherichia coli and Pseudomonas aeruginosa were detected in clinical isolates of Acanthamoeba[22]. Some microorganisms such as Staphylococcus aureus and P. aeruginosa can form microfilms on contact lenses, leading to Acanthamoeba keratitis by interactions with Acanthamoeba. P. aeruginosa can lead to more adhesion of Acanthamoeba by creating certain conditions in biofilms, which ultimately leads to keratitis[12, 23-26].

Biofilms can be formed in different places, including contact lenses, case of contact lenses, and multipurpose contact lens cleaning solutions (MPS). It is essential to mention that overnight lens wearers are at higher risk than the rest[27,28]. Higher risk is due to more corneal exposure to infection source, and lens overuse caused hypoxia. Under these conditions, microorganisms can invade the cornea, colonize on its surface, and form a biofilm that eventually leads to corneal damage and Acanthamoeba keratitis.

There are certain substances in teardrops, such as mucins, lysozyme, and lactoferrin, which can deposit inside the contact lens and facilitate the adhesion of microorganisms, including Acanthamoeba, to the surface contact lenses[29]. Tear compounds can reduce the culture of P. aeruginosa[30].

Biofilm and MPS: Another critical issue is the accumulation of bacteria, fungi, and Acanthamoeba in the MPS. These can be transmitted to the MPS through the surface of infected cases. Acanthamoeba in MPS biofilms can easily feed on bacteria. It is important to note that MPS induces encystation and resistance of Acanthamoeba, so wearing the lens can result in keratitis4. It needs to consider MPS and eye-wash solutions standardization tests are established using planktonic strains and all cells and microorganisms that are very sensitive to antibiotic substances. It is important to note that the physiological states of microorganisms and different species of microorganisms must be considered; as mentioned, Acanthamoeba may be resistant to these solutions on biofilms, and it can easily survive in solutions and enter the eye through lenses[4, 31].

Prevention of biofilm formation: There are several ways to prevent biofilm formation, two of which are mentioned following:

Covalently bonding of biocidal molecules on the surface of contact lenses;

The slow-release of antibiotics in contact lenses can be one of the most critical factors preventing biofilm formation. Rifampicin and clarithromycin are among the slow-release antibiotics that can prevent the in vitro formation of biofilms for up to 3 weeks[32].

Also, antimicrobial peptides can successfully prevent biofilm formation. Significant efficacy against antibiotic-resistant strains is an important advantage of these peptides[33-35].

Another essential issue to note is that improving the quality of ophthalmic devices, implants, and lenses can reduce the adhesion and attachment of microorganisms to the surface of the lens and prevent their colonization. If this happens, the need to attach and coat the antibiotics on the surface of the lens will be less[36].

Antimicrobial agents, antifungal agents, and some antimicrobial peptides are coated on the inside surface of the lens. The coated lens is placed on a healthy eye that prevents the accumulation of microorganisms and eliminates the colonized microorganisms.

Conclusion

In conclusion, contact lenses are essential in improving people's vision, so people are willing to use contact lenses. However, due to possible contaminants on the surface of the lenses, their use is limited. As mentioned, to form a biofilm, the planktonic cells are placed individually on the surface, secrete a series of polysaccharide and peptide materials inside this surface, and make a strong connection, so they are no longer separated. Among the various microorganisms that participate in the formation of biofilms in contact lenses and cause keratitis, various strains of *Staphylococcus* and *Pseudomonas* were mentioned. It was also noted that *P. aeruginosa* in interaction with *Acanthamoeba* could lead to more robust bonds in the environment and eventually *Acanthamoeba* keratitis. Intense interaction between the contact lens and the corneal epithelium can result in hypoxia, colonization of microorganisms and the formation of biofilms on the surface of the cornea, and eventually corneal damage.

On the other hand, it was also addressed that MPS are critical. So, to ensure that these solutions can kill all the microorganisms that make up the biofilm and prevent the formation of new biofilms, different physiological states of microorganisms should be considered in their standardization tests. Finally, it was pointed out that antimicrobial coating materials on the surface of contact lenses can effectively treat keratitis and prevent the formation of biofilms. The attention of researchers to this may reduce biofilm-related eye infections associated with contact lenses in the future.

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Conflict of interest

The authors further declare that, they have no conflict of interest.

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