



Original Research Article

Antiadhesive role of multipurpose solutions in prevention of ocular infections: an invitro study

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ABSTRACT

Keywords

Biofilm,
contact lens,
Multi Purpose
Solution
(MPS),
antiadhesive.

Considering the number of growing ocular problems in Mumbai city due to increase in the usage of contact lens for vision correction and for increasing aesthetic appeal, the present work was aimed at carrying out an invitro study of the biofilm formation on the surface of contact lenses. Two isolates obtained from the surface of contaminated contact lenses, namely *Candida albicans* and *Pseudomonas aeruginosa* were used in the study. The biofilm formation on the surface of contact lenses was studied quantitatively by measuring its dry weight as well as by Tetrazolium Reduction Assay. A study of the anti-adhesive efficacy of one Multi Purpose Solution (MPS) namely, ReNu on the biofilm development of *Pseudomonas aeruginosa* was also studied qualitatively using Scanning Electron Microscopy SEM.

Introduction

Contact lenses remain majorly popular among individuals who need to add on to the aesthetic appeal as well as help in vision correction (Willcox and Holden, 2001). But, contact lens wearers are prone to development of microbial keratitis because lenses are a potential means of transport of microorganisms to the cornea. Contact lens surface has to be kept sterile during the time of wearing but poor patient compliance with lens wear schedules and care regimens has been a major contributory factor to eye infections. These contact lens surfaces are prone to colonization by various types of bacteria,

fungi, virus and protozoa. These environments become more and more complex with age, time of wearing, user's sensitivity for contact lenses and quality of ocular hygiene. Certain other lipid secretions from the eye glands (meibomian glands) can also bind to the lens surface, forming a lipoprotein film that is very difficult to remove. All such deposits if not removed then may cause discomfort and impair visual acuity. Microorganisms may further build up on these deposits and the situation further worsens (Arora *et al.*, 2009). Continuous contact lens wearing and poor patient compliance for contact lenses helps ocular organism to persist and

multiply resulting in formation of biofilm on the contact lens. Biofilms form large microbial communities on the lens surface and have coordinated multi-cellular behavior, which can confound the medical and optical properties of the lens. Microbial biofilm so formed can lead to various diseases like keratitis and endophthalmitis. *Pseudomonas* species are the major ocular pathogens posing significant health risk if they are able to form biofilm on the surface of contact lenses as they can lead to further serious complications like Giant Papillary Conjunctivitis (GPC) which may lead to permanent vision loss (Bruinsma *et al.*, 2002). Following the primary invaders, the ocular lenses become vulnerable for secondary invaders like *Candida albicans*, *S.aureus*, *S.mutans*, *Klebsiella pneumoniae* etc. (Brooks *et al.*, 2001; Sankaridurg *et al.*, 1996; Slusher *et al.*, 1987 as cited in Nwaugo *et al.*, 2008) Physical action on contact lens in order to clean the lens is not enough to remove adherent bacteria on contact lens. An antimicrobial and anti-adhesive agent in cleaning solution inhibits biofilm formation thus, preventing further complicated corneal infections. In addition cleaning solutions also contain lubricants, moisturizers, and wettability enhancers found to be beneficial for good ocular health. Such an agent also enhances patient compliance for contact lenses. Various Multipurpose solutions (MPS) available in the local market include imported solutions claim to be most efficient protein and lipid deposit cleaner as well as antimicrobials. One of the functions of MPS is to remove lens deposits when lenses are soaked in the solution overnight. In this way, it extends the useful life of the lens and keeps the lens free from deposits and thereby provides clear vision, comfort and

maintain normal eye health (Arora *et al.*, 2009). The present study was therefore aimed at invitro qualitative and quantitative study of the development of biofilms on the contact lens surface by isolates viz. *Candida albicans* and *Pseudomonas aeruginosa* obtained from such contaminated lens surfaces and a study of the antiadhesive efficacy of one such commercially obtained MPS from the local markets, namely ReNu.

Materials and Methods

Quantitative Measurement of biofilm growth by measurement of dry weight of biofilm and Tetrazolium Reduction Assay

80 µl cell suspension of individual isolates viz. *Candida albicans* and *Pseudomonas aeruginosa* (10^8 cells / ml) was separately applied to the surface of sterile contact lenses placed in sterile glass tubes respectively. Each lens was then submerged in 1ml Yeast Nitrogen Base Medium containing 50 mM glucose and incubated at $36\pm 1^\circ\text{C}$ for 1 h, 2 h, 24 h, and 48 h respectively. A control was setup where lenses without any inoculum were incubated in the same nutrient medium. The tubes were set up in duplicates. After the stipulated incubation period, one tube was used to measure the biofilm formed on the surface of the contact lenses in terms of its dry weight. Simultaneously, to the second tube, 0.2% MTT i.e. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl Tetrazolium bromide solution was added to each tube and further incubated for 5 h at $36\pm 1^\circ\text{C}$. The medium plus MTT was removed and the lenses were washed three times with 2 ml PBS (0.15 M) to remove all traces of MTT. Acetone was added to solubilize the MTT formazan product which was then measured at 540

nm by using a Shimadzu Spectrophotometer. A control containing medium plus MTT was used to determine background formazan values..

Contact lens adherence assay and Scanning Electron Microscopy

(Kodjikian *et al*; 2003).

Two test tubes containing 10 ml sterile Balanced Salt Solution were taken into which a contact lens each was introduced. The first tube was inoculated with 18 h old *Pseudomonas aeruginosa* (10^8 to 10^9 cells/ ml), whereas in the second tube along with *Pseudomonas aeruginosa*, 1 ml solution of ReNu Multipurpose solution was also added. The lenses were incubated in the bacterial suspension for 1 h. at 36 ± 1 °C, with continuous shaking (180 rpm). All the samples were then scanned using Scanning Electron Microscopy.

Results and Discussion

Biofilm growth for both the culture isolates on the surface of contact lens material was studied in terms of conversion of yellow tetrazolium salt, MTT to a dark pink product, MTT formazan as a result of the dehydrogenase activity by biofilm cells which was measured spectrophotometrically at a wavelength of 540 nm. The study showed an excellent correlation between biofilm dry weights. Growth of biofilms of *C. albicans* in medium reached a maximum after 48 h and then declined, possibly because of the depletion of oxygen or nutrients within the biofilm (Fig 1). The growth pattern for *Pseudomonas aeruginosa* was also quite similar (Fig 2). Biofilm formation depends on the ability of the bacteria and yeast initially to attach to the solid surface and on the production of extracellular polymeric material in

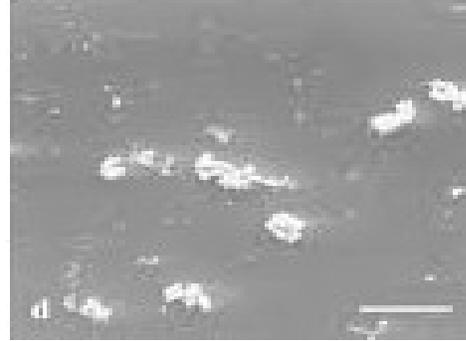
which the cells become embedded. In *C. albicans* and *Pseudomonas aeruginosa*, both these properties are affected by the nature and concentration of the available carbon source. Growth in medium containing nutrient promotes the synthesis of the fibrillar mannoprotein, adhesin resulting in increased yeast adhesion to epithelial cells and inert surface. Prolonged incubation in this medium causes release of the fibrils and extracellular polymeric material can be isolated from culture supernatant fluid. Presumably, this extracellular material could function as matrix substances in biofilms. The pathogenesis of *P. aeruginosa* in keratitis is attributed to mucoid phenotype caused by overproduction of alginate, an exopolysaccharide (EPS). Fully developed biofilms exhibit a highly heterogeneous architecture composed of cellular and non-cellular elements. This biofilm formation leads to many ocular infections like endophthalmitis and keratitis. MPS solutions have surfactants which help their solution to remove dirt particles along with protein and lipid deposition on the contact lenses. The lubricating property of the surfactant which leads to formation of a layer around the contact lens helps to prevent the adherence of microorganism on the surface of the lens and inhibits biofilm formation by organisms like *Pseudomonas aeruginosa*. Furthermore, antimicrobial agents present in MPS solution also act to inhibit growth of the microorganisms.

In this present investigation, a comparative study of the ability of *Pseudomonas aeruginosa* to form biofilm on the contact lens was carried out in presence and absence of ReNu (MPS) solution. Biofilm formations along with various changes taking place on the lens were studied using

Plate.1 Photographs and graphical representations of the results



A) SEM of *Pseudomonas aeruginosa* biofilm on contact lens without treatment solution of MPS solution



B) SEM of *Pseudomonas aeruginosa* biofilm on contact lens after treatment with MPS

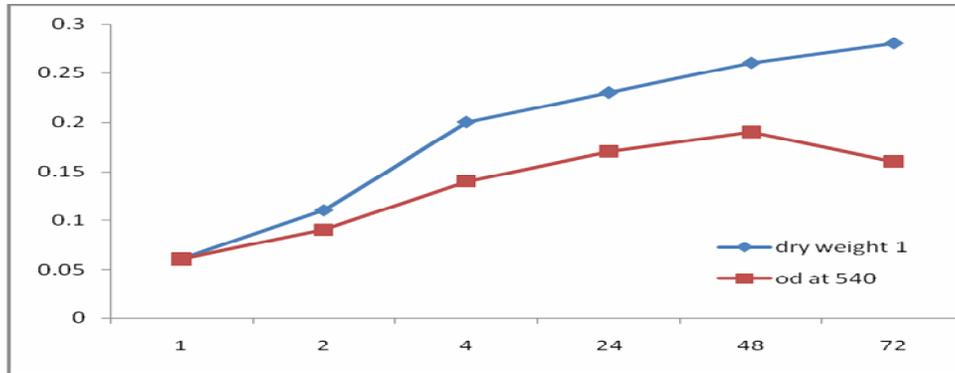


Fig.1 Biofilm formation of *Candida albicans*

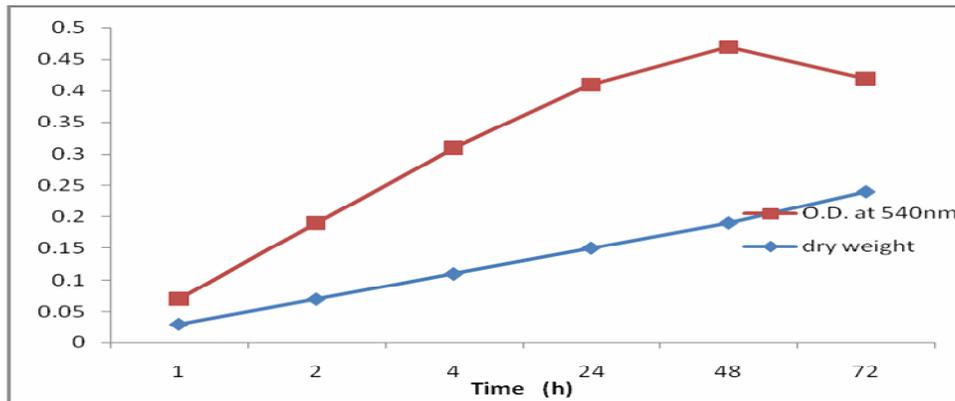


Fig.2 Biofilm formation of *Pseudomonas aeruginosa*

Scanning Electron Microscopic analysis of the same. Bacterial adherence to contact lens is a primary requirement in the initiation of ocular infection. Among many ocular bacterial species examined *Pseudomonas aeruginosa* has been suggested to be a major causative agent of ocular infections. The ability of *Pseudomonas aeruginosa* to colonize eye lens could be ascribed to its ability to synthesis of exo- polysaccharides which form a confluent matrix around colonies. The scanning electron microscopic analysis of the colony formation on the contact lenses using *Pseudomonas aeruginosa* was thus in corroboration with the earlier results.

The present study has demonstrated that the associated microorganism forms an extensive network such that the mature biofilm comprise all morphologies of the present microorganism embedded in an exopolymeric matrix material. The cells were found to synthesize a large quantity of gelatinous polysaccharides when strain of *Pseudomonas aeruginosa* was cultivated separately, in the presence and in the absence of the MPS (Plate 1 and 2). This study also indicated that in the absence of MPS, the biofilm formed was more homogeneous with cell embedded in extracellular polysaccharides, due to which the structure of cells were seen prominently. Abundant amorphous extracellular structure were seen which confirms that the surface of the *Pseudomonas aeruginosa* and providing support to the adherence. Based on the SEM images, the predominant morphology of *Pseudomonas aeruginosa* cells when cultivated in the absence of MPS was homogeneous and showed exopolymeric growth. But in the presence of MPS this exopolymeric growth was formed inhibited. This may be due to the lubricating property of surfactant i.e. Poloxamine (1%) present in the MPS. This must have inhibited biofilm formation on the surface of contact lenses.

From this result we can conclude that *Pseudomonas aeruginosa* has efficient adherence mechanism, but in the presence MPS this efficient mechanism does not result in biofilm formation.

Contact lens wearers are prone to development of microbial keratitis because lenses are a potential means of transport of microorganisms to the cornea. The invitro study was thus successful in demonstrating the ability of a multipurpose solution for inhibiting the adherence and further biofilm development by ocular pathogens like *Pseudomonas aeruginosa* and *Candida albicans* on the surface of contact lenses thus preventing infections by these organisms.

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