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The effects of human tear electrolytes on the viability and hydrogel colonization of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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This study was designed to evaluate the effects of the inorganic electrolytes present in human tear film on the viability and colonization of bacteria to hydrogels. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used in these experiments. A *D*-value test was performed to investigate any bacteriostatic effect by measuring the reduction of viable test microorganisms over time when exposed to the inorganic electrolyte solution. No *D*-value was calculable for *S. aureus* in electrolyte solution whereas a *D*-value of 8.1 h was obtained for *P. aeruginosa* in electrolyte solution. The *D*-value data indicate that staphylococci have a greater survivability potential in a hypertonic environment than do pseudomonads. Bacterial adhesion to high water, ionic hydrogels was studied using the Modified Robbins Device (MRD). The data for *P. aeruginosa* recovered from the lenses showed an 82% decrease in bacterial counts in electrolyte solution as compared to bacteria incubated in control solution. In contrast there were slight increases in *S. aureus* counts recovered from the lenses. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 17-19.

Keywords: electrolyte; bacteria; *S. aureus*; *P. aeruginosa*; adhesion; bactericidal

Introduction

The inorganic electrolytes in the human tear film serve many physiological functions in the ocular environment. The osmotic pressure of human tears, typically about 329 mOsm/kg [12] is regulated by ions, such as, Na^+, Cl^-, HCO_3^-, and K^+ [11]. Maintenance of the proper tonicity sustains the epithelium by controlling water flow from the corneal and conjunctival surfaces to the tear film [2,13]. Also, Ca^{2+} ions play roles in cell adhesion, glycogen metabolism, removal of exfoliated cells [9], as well as inhibition of oxygen free radical formation [11]. In addition, various ions, e.g. iron, copper, magnesium and calcium serve as cofactors for enzymes [8]. Several corneal degeneration diseases, such as keratoconjunctivitis sicca and Sjögrens syndrome, result from deficiencies in the aqueous phase in human tears [5].

As a corollary to this, bacterial adhesion to contact lenses has been a source of industrial interest since the advent of the medical device. Various investigators have studied adhesion patterns to both worn and unworn contact lenses [1,4,6]. However, the relationship between the contact lens and bacteria in the ocular environment is poorly understood. Experiments outlined in this paper were designed to determine if the inorganic electrolyte component of human tears affects the kinetics of bacterial presence on the hydrogel matrix.

Materials and methods

Preparation of the test microorganisms

The microorganisms evaluated were *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 6538 (American Type Culture Collection, Manassas, VA). *P. aeruginosa* 15442 was isolated from an animal room water bottle and is a challenge microorganism for the multi-item test (MIT) for evaluating contact lens disinfecting solutions. *S. aureus* 6538 was isolated from a human lesion, and is representative of the species most commonly encountered in ocular infections [10].

P. aeruginosa and *S. aureus* were subcultured in soybean casein digest broth (TSB) (Dieco, Detroit, MI) and incubated at $37\pm1^{\circ}C$ for 18–24 h in ambient air. A standardized inoculum for each test microorganism was prepared by obtaining a percent transmittance (%*T*) reading that corresponded to about 1×10^8 CFU/ml.

Preparation of the test solution

The formulation of the electrolyte solution (ES) was based on published levels of inorganic salts in human tears [6,7,15]. The electrolyte solution contained (in mM): Na^+ 130, Cl^- 135, HCO_3^- 23, Mg^{2+} 0.7, K^+ 31 and Ca^{2+} 0.8 for a total osmolarity of 320.5 mOsm/l.

The saline solution (SS) was prepared by adding 9.0 g NaCl into 1 l of 18 Ω reverse osmosis water. The pH of ES and SS was adjusted to 7.4 using either 1 M HCl or 1 M NaOH (both from Sigma, St. Louis, MO). The ES and SS were then filter (0.22 mm) sterilized. The purity of the inorganic salts was ≥99%.

Bacteriostasis/*D*-value test

Fifty milliliters of ES and SS were aseptically transferred into two separate Erlenmeyer flasks each containing bacterial suspensions of *P. aeruginosa* and *S. aureus* of approximately 1×10^6 CFU/ml (5×10^7 CFU/50 ml). Sampling times for bacteria in both solutions were 0, 1, 3 and 6 h. To ensure a homogenous sample each flask was mixed on a stir plate (Baxter, Deerfield, IL) prior to sampling.

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Table 1 Average recoveries (CFU/ml) of *P. aeruginosa*, ATCC 15442 from ES and SS control at each time interval

Solution	ES	SS control
0 h	2.2×10^6	2.2×10^6
1 h	1.9×10^6	2.3×10^6
3 h	7.4×10^5	3.5×10^6
6 h	4.0×10^5	5.7×10^6

Bacterial adhesion assay using the MRD

The Modified Robbins Device (MRD) (MicroBios, Ltd., Calgary, Alberta, Canada); an apparatus that allows surfaces, e.g. lens polymer matrices, to be exposed to a recirculated suspension of viable microorganisms, was used as previously described [14]. Three sterile high-water, ionic contact lenses were aseptically transferred into a conical test tube and washed three times with 30 ml of SS for about 10 min per wash to rinse residual packing solution. Each lens was then cut at 120° using a straight razor blade and a domed cutting surface to produce three equivalent v-shaped sections. The resulting lens sections were affixed to separate 0.5-cm^3 rubber backings as previously described [14]. Sterile ES (495 ml) was aseptically transferred into the sterile 1–2 side-arm flask. The inoculum concentration was 2.9×10^4 CFU/ml.

Calculations

Following incubation all plates were enumerated, recorded, and the appropriate calculations performed.

The D-values for ES were calculated using Stumbo's equation [16]:

$$D = T_X - T_O / \log N_O - \log N_X,$$

where: T_X=ending time (h), T_O=initial time (h), N_O=CFU/ml present at initial time, N_X=CFU/ml present at end of time interval.

The time point selected as N_X was the first sample point to demonstrate a minimum of one $\log_{10}$ reduction of the test microorganism. If no reduction or less than one $\log_{10}$ was observed the 6-h sample time was used to determine the D-value.

Results

Bacteriostasis/D-value test

The inoculum concentrations of *P. aeruginosa* and *S. aureus* were 3.8×10^8 and 6.7×10^7 CFU/ml, respectively.

Table 1 shows the average viable recoveries (CFU/ml) of *P. aeruginosa* from ES and SS at each time interval. Viable counts of *P. aeruginosa* fell from 2.2×10^6 to 4.0×10^5 CFU/ml (82% decrease) over a 6-h exposure time in ES. Bacteria incubated in the SS control displayed a slight increase in cell counts from 2.2×10^6 to 5.7×10^6 CFU/ml.

Table 2 Average recoveries (CFU/ml) of *S. aureus*, ATCC 6538 from ES and SS control at each time interval

Solution	ES	SS control
0 h	7.7×10^5	7.0×10^5
1 h	1.4×10^6	1.4×10^6
3 h	4.1×10^6	1.1×10^6
6 h	7.1×10^6	1.0×10^6

Table 3 Calculated $D_{37°C}$ (hours) for test microorganisms in ES and SS control

Test microorganism	ES	SS control
P. aeruginosa, ATCC 15442	8.1	none
S. aureus, ATCC 6538	none	none

The average viable recoveries (CFU/ml) of *S. aureus* from ES and SS at each time interval are displayed in Table 2. Viable counts of *S. aureus* increased slightly in both ES and SS from 7.7×10^5 and 7.0×10^5 CFU/ml to 7.1×10^6 and 1.0×10^6 CFU/ml, respectively. There was no $D_{37°C}$ for *S. aureus* in ES as no reduction of the test microorganism was observed over the 6-h exposure period. The calculated $D_{37°C}$ for *P. aeruginosa* in ES was 8.1 h (Table 3).

Bacterial adhesion assay using the MRD

P. aeruginosa and *S. aureus* inocula were 1.8×10^8 and 2.9×10^8 CFU/ml, respectively. The average recoveries (mean±standard deviation) of viable *S. aureus* from the lens sections of the three test lenses were 300±160, 350±90, and 240±31 CFU/ml with an overall mean and standard deviation of 300±110 CFU/ml. The 0.5-cm^3 rubber backings adhered an average of 20 CFU/cm^3. The initial inoculum of *S. aureus* showed an increase in concentration from 2.9×10^4 to 1.6×10^5 CFU/ml over the 4-h exposure to ES. *P. aeruginosa* did not adhere to contact lens sections in numbers exceeding 10 CFU/ml after a 4-h exposure time to ES. These results may be explained by the reduction of the initial inoculum from 1.8×10^3 to 6.7×10^2 CFU/ml over the 4-h exposure time, an effect demonstrated in the D-value test.

Discussion

P. aeruginosa ATCC 15442 decreased over time in the presence of electrolyte solution (ES) ($D_{37°C}$, 8.1 h). This reduction of viable *P. aeruginosa* may be caused by the osmolarity of ES. In the MRD adhesion assay, the viability of the test microorganism is critical for an accurate measure of colonization for the adhesion rate is a function of cell concentration. Therefore, no conclusion could be drawn about *P. aeruginosa* adhesion to contact lenses in the presence of ES as the colonization decreased below measurable levels.

In contrast, *S. aureus* ATCC 6538 increased in concentration over time in the presence of ES. As a result, no D-value was calculable. The survival of *S. aureus* in hyperosmotic environments is well characterized in the literature [3], so these results are not unexpected. The ES component of the human tear film may select for *Staphylococcus* spp. as the predominant natural flora isolated from the ocular environment. Therefore, the viability of *S. aureus* did not interfere with the assessment of colonization to the contact lenses in the presence of ES.

The data obtained by these experiments imply that different microorganisms will grow or be inhibited by physiological concentrations of tear electrolytes and that their respective hydrogel colonization is directly proportional to the concentration of viable microorganisms present. Since various bacterial species, including Gram-negative microorganisms can exist in the human

tear film, it is clear that the effects of the electrolyte component individually must be somewhat minimized by the remaining components. However, if the homeostatic regulation of the tear film should decline and the tear profile shift, the potential for unbalancing this homeostatic regulation may increase. For example, in dry eye disorders, such as keratoconjunctivitis sicca and Sjögren's syndrome, the tear film is characterized by elevated concentrations of electrolytes due to reduced aqueous secretions by the lacrimal gland and evaporation of the existing tear film [5]. In this hyperosmotic environment, *S. aureus* would have an increased opportunistic advantage in the presence of a corneal abrasion.

In summary, the effects of the inorganic electrolytes in human tears on the viability and hydrogel colonization of *P. aeruginosa* and *S. aureus* were investigated. *P. aeruginosa* adhesion to hydrogels was adversely effected by the ES because of the viability of the test microorganism. However, bacterial counts were demonstrated for *S. aureus* adhesion to contact lenses in ES. The ES results obtained in these experiments were in concert with data for other tear components, e.g. lipids, proteins, or organic solutes might elucidate the tear component predominantly responsible for bacterial adhesion. The inorganic components in tear film may act with these other organic molecules to control the growth of bacteria in the ocular environment in the absence of an epithelial break that would lead to a disease state.

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