Lens material and formulation of multipurpose solutions affects contact lens disinfection

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ARTICLE INFO

Keywords:

Contact lens disinfection
Multipurpose lens care solutions

ABSTRACT

Purpose: To assess the disinfection efficacy of multipurpose solutions (MPS) against different bacterial species adhered either to silicon hydrogel or to conventional hydrogel contact lenses (CLs). The influences of the MPS formulation and the chemical composition of the lens material were investigated.

Methods: This investigation followed the standard 14729, which establishes the guidelines for assessing CL disinfecting solutions. Two commercially available (Opti-Free® Express® and Renu® Multiplus) solutions and one recalled solution (Complete® MoisturePlus™) were used in this study. After disinfection, the number of survivors was estimated by the colony forming units' method.

Results: The lens material appears to influence disinfection. The conventional hydrogel polymacon exhibited the highest disinfection scores, a fact that should be related with the lack of electrostatic attraction towards the biocides and its hydrophilicity. The MPS formulation appears to have influence in disinfection efficacy as well. For most adhered bacteria, Opti-Free® was capable of reducing cell concentration in 4-log.

Conclusion: Disinfection results from multivariate factors and this study confirmed that the lens material and the MPS play a very important role in the disinfection efficacy of CL.

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1. Introduction

The presence of microorganisms on contact lenses (CL) is associated with infiltrative keratitis (IK) an inflammatory condition, and microbial keratitis (MK) an eye infection that might ultimately lead to vision loss [1]. The factors involved in the occurrence of IK are not entirely understood; however, it is hypothesized that continuous wear, the presence of deposits, and bacteria and toxins adherent to the lens are important etiological factors [2]. With concern to MK, it is well established that poor hygiene and continuous wearing schedules are risk factors. The most common etiological agents isolated from CL-associated MK are *Pseudomonas aeruginosa* and *Staphylococcus* spp. [3–10]. In order to minimize the risk of either inflammatory as infectious events associated with lens wear, the use of disinfecting solutions is recommended, as they enable the removal of deposits and inactivate bacteria and respective toxin production [11].

Being true that the incidence of IK and MK could be reduced by using MPS it is also true that some formulations are not compatible with some lens materials and as a result may affect the cornea integrity, cause discomfort, inflammatory events and even-

tually discontinuation of lens wear [12,13]. So, clinicians should pay special attention to compatibility between lens materials and MPS when prescribing a disinfecting solution.

MPS can be roughly separated into 2 groups: the group containing the biocide biguanide such as Renu® Multiplus or Complete® and the one containing polyquaternarium, such as Opti-Free®. Conventional hydrogel CLs are made of hydrophilic monomers and present a smooth and wettable surface [14,15] whereas silicone hydrogel CLs are made from hydrogel monomers and silicone elastomers, resulting in a hydrophobic material [16]. In order to increase their wettability, the surface of these lenses can undergo a plasma treatment [16].

Disinfection of MPS on silicone hydrogel CL is poorly understood, first because MPS were designed for a different class of lenses, conventional hydrogel CLs, and second because the standard 14729 used to evaluate the disinfection efficacy of MPS has an important flaw: the primary criteria are made against suspended microbes not microbes adhered to CLs [17]. In fact, CL disinfection is a complex process as it results from multiple interactions. Different microbial strains exhibit distinct susceptibilities to biocides [18,19], adhered microbes are more resistance to biocides than in planktonic state [20,21], and exopolysaccharides protect microbes against the biocidal action [21]. The presence or absence of tear film affects disinfection; therefore, worn lenses may exhibit different disinfection scores than unworn ones [22]. Other important

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Table 1

Properties of the multipurpose solution enrolled in this study.

Product	Manufacturer	Biocide (%)	Surfactant cleaner	EDTA (%)
Opti-Free® Express®	Alcon	PQ-1 0.001 MAPD 0.0005	Poloxamine	0.5
Renu® Multiplus	Bausch & Lomb	PHMB 0.0001	Poloxamine	0.1
Complete® Comfort Plus	AMO	PHMB 0.0001	Poloxamer	0.02

PQ-1, polyquaternium-1; PHMB, polyhexamethylene biguanide; MAPD, myristamidopropyl dimethylamine; EDTA, ethylenediamine tetraacetic acid.

feature concerns the biocide uptake. Either CL materials as lens case are capable of uptaking the biocide present at the MPS thus leading to the overall reduction of its disinfection efficacy [23]. The most recent ISO and FDA standards ignore these facts, which may very well contribute to the past infection outbreaks and product recalls. Currently, new standards are being formulated, so improvements are expected in the way disinfection is assessed.

In the present work it is hypothesized that different MPS formulations exhibit different scores of disinfection on contaminated CL. Therefore several MPS will be tested against 4 CL materials contaminated with two Gram-positive and one Gram-negative strain. The aim is to assess the influence of the MPS formulation and the influence that CL physic-chemical properties might exert upon disinfection.

2. Materials and methods

2.1. Chemicals and culture media

Table 1 details the properties of the MPS used in this study. Three MPS with distinct formulations were tested. Opti-Free® Express® contains two biocides, PQ-1 and myristamidopropyl dimethylamine (MAPD), while Renu® Multiplus and Complete® MoisturePlus™ incorporate PHMB. Two lots from each MPS were assayed.

Tryptic soy agar (TSA) and Dey-Engley-Neutralizing Broth (DENB) were purchased from Sigma–Aldrich, Germany. Dulbecco's phosphate buffered saline (DPBST) included 8.0 g l⁻¹ NaCl, 0.2 g l⁻¹ KCl, 1.15 g l⁻¹ Na₂HPO₄, 0.2 g l⁻¹ KH₂PO₄, and 0.05 (w/v) Polysorbate 80.

2.2. Contact lenses

Table 2 presents the CLs used in this work: two-silicone hydrogel CLs and two conventional hydrogel CLs were enrolled in this study. Both silicone hydrogel CL are surface treated.

2.3. Test organisms and growth conditions

Three bacterial species were used in this study. *Staphylococcus aureus* CECT (Colección Española de Cultivos Tipo) 239 and *Pseudomonas aeruginosa* CECT 111 were purchased from the Spanish Collection of Type Culture, University of Valencia. These strains are equivalent to *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 9027, respectively, of the American Type Culture Collection [24]. Although not considered a reference microorganism, *Staphylococcus epidermidis* 9142 was used. This bacterium is a clinical isolate

and a producer of the major surface polysaccharide promoting coagulase-negative staphylococci adherence and biofilm formation, referred to as either polysaccharide intercellular adhesin (PIA) or, by its chemical composition, poly-N-acetyl glucosamine (PNAG), and its adhesion capability has been characterised previously [25,26]. This strain was kindly provided by Dr. Gerald B. Pier, Harvard Medical School, Boston, USA.

Bacteria were grown on agar slopes for 18 h at 35 °C. The colonies were harvested with DPBST which contains Polysorbate 80. This surfactant aids the harvesting process as well as cell dispersion. The suspension was vortexed for 1 min in order to ensure complete homogenization and transferred to a sterile polypropylene tube (Orange Scientific, Belgium). Owing to its voluntary recall, Complete® MoisturePlus™ was not challenged against *P. aeruginosa* CECT 111.

2.4. Disinfection tests against bacteria adherent on contact lenses

The protocol followed the standard ISO 14729 for regimen test; however a modification was made. While the standard dictates that each side of the lens should be inoculated with 10 µl inoculums, which is not enough to cover the lens surface, in this work a 100 µl inoculums was placed in each side of the lens, resulting in the coverage of the entire lens surface and as consequence a greater bacterial load. According to the standard, the bacterial concentration of each lens should be comprised between 5 × 10⁵ CFU/lens and 5 × 10⁶ CFU/lens. Being true that this concentration is appropriated to undertake this test, it does not necessarily reflect other scenarios where lens wearers overwear their lenses or skip disinfection resulting in greater levels of microbial load. Keeping this in mind, lenses were inoculated with a more demanding bacterial concentration.

A bacterial suspension was prepared as previously described in Section 2.3. The suspension was dipped on both the concave and convex sides of the lens, 100 µl in each side. After 10 min exposure to the bacterial suspension, excess was removed. Each CL had a concentration between 1.0 × 10⁷ and 1.0 × 10⁸ CFU per lens.

Each lens was transferred to a well of a 24-well culture plate containing 1 ml of the test MPS. Lenses were soaked for 4 h at room temperature. After soaking, the lenses and the soaking solution were poured to a filtration apparatus containing 50 ml DENB in order to inactivate the disinfectants. After 15 min of neutralization, three filtrations containing 50 ml DENB were made using 0.2 µm sterile nitrocellulose membranes. Each CL was placed in an eppendorf containing 1 ml DENB and vortexed for 1 min to detach adhered bacteria. Serial dilutions were carried out in DENB and spread on TSA plates. After incubation (48 h at 35 °C), the number of viable

Table 2

Properties of the contact lenses enrolled in this study.

CL material	Brand	Manufacturer	Ionicity	Water content (%)	Principal monomers
Balafilcon A	Purevision™	Bausch & Lomb, Inc.	Ionic	36	NVP + TPVC + NCVE + PBVC
Lotrafilcon A	Focus® Night & Day™	CIBA Vision Corporation	Non ionic	24	DMA + TRIS + siloxane monomer
Polymacon	Soflens 38	Bausch & Lomb, Inc	Non ionic	38	HEMA
Etafilcon A	Acuvue2®	Johnson & Johnson Vision Care	Ionic	58	HEMA + MA

HEMA, 2-hydroxyethyl methacrylate; mPDMS, monofunctional polydimethylmethacrylate; NVP, N-vinyl pyrrolidone; TPVC, tris(trimethylsiloxy)silyl propylvinyl carbamate; NCVE, N-carboxyvinyl ester; PBVC, (poly[dimethylsiloxy]di[silylbutanol]bis[vinyl carbamate]; DMA, N,N-dimethylacrylamide; TRIS, trimethylsiloxane.

Table 3

Log reduction units of adherent bacteria to contact lenses.

	Balafilcon A	Lotrafilcon A	Polymacon	Etafilcon A
<i>S. epidermidis</i>				
Opti-Free®	3.18	6.54*	6.64*	5.20*
Complete®	2.43	2.18	4.23*	3.20
Renu®	1.96	2.06	4.32*	3.94
<i>S. aureus</i>				
Opti-Free®	4.16*	4.28*	4.30*	4.18*
Complete®	2.28	1.12	4.19*	2.18
Renu®	2.03	2.83	3.38	1.80
<i>P. aeruginosa</i>				
Opti-Free®	5.44*	3.38	5.74*	5.16*
Renu®	3.86	1.64	6.04*	3.58

* Statistically different from the other combinations (Mann–Whitney's *U* with 95% of confidence level).

cells was counted by CFUs. At least three replicates and two independent assays were performed for each combination of CL, MPS and bacterial strain. The control condition for each experiment was the CL exposed to a bacterial suspension prepared in saline solution.

2.5. Data analysis

The data distribution was analyzed using the Kolmogorov–Smirnov test and distribution was shown to be 'not normal'. Therefore, the non-parametric Mann–Whitney *U* test was employed, with a 95% confidence level. Comparisons were made between CL material and MPS. A *p* value of less than 0.05 was considered statistically significant.

3. Results

The data presented in Table 3 show that different combinations of MPS and CL result in distinct rates of disinfection. Polymacon has given uniformly better log unit reduction than the other lens types. For example, the log reduction of *S. epidermidis* adherent to balafilcon A, ranged between 1.96 and 3.18, while in the case of polymacon varied between 4.23 and 6.64. Also in polymacon, the log-reduction units found for *S. aureus*, ranged between 3.38 and 4.30 and regarding adherent *P. aeruginosa* between 5.74 and 6.04. These values were significantly superior to those found for the other tested materials ($p > 0.05$).

After the neutralisation process, lenses were sandwiched on agar plates and incubated. As shown in Table 4, the number of survivors on lenses treated with Opti-Free® was less than 22 CFU/per lens, while lenses treated PHBM-based MPS displayed a number of CFUs so excessive that could not possible to enumerate through this counting technique. These results (Tables 3 and 4) suggest that combinations involving polymacon and Opti-Free®, are the ones giving uniformly better log unit reduction.

4. Discussion

The present study evidences that different combinations of MPS and CL result in different levels of lens disinfection (Table 3). Such differences may be attributed to different degrees of biocide uptake

onto the lens matrix. The fact is that when a biocide and lens material interact to sequester the biocide within the lens matrix, the system loses efficacy. This phenomenon is reported in the literature [23,27–30]; however, this is the first time that disinfection is quantified for such a wide variety of CL materials and MPS.

Contemporary ISO and FDA standards virtually ignore the biocide uptake phenomena albeit its importance on disinfection efficacy; however, during the first advisory board following the recall of two MPS it was hypothesized that biocide uptake could have contributed to the inefficacy of the recalled solutions.

The phenomenon of biocide uptake is ruled by the interaction generated between the CL material and the biocide and also by the hydrophobicity of either biocide as the material [31,32]. An example of the first situation is the electrostatic attraction that occurs when materials and biocides have opposite charges [32]. It was previously described that the conventional hydrogel etafilcon A, has greater propensity for biocide uptake [27] a fact that was attributed to an electrostatic attraction established between the biocides PHMB, PQ-1 and MAPD all having cationic domains and etafilcon A which is negatively charged. On silicone hydrogel the biocide uptake might be driven not only by electrostatic attractions, but also by hydrophobic interactions. Due to their hydrophobic nature these materials are capable of establishing attractive forces with the nonpolar heads of the biocides, and to sequester them [31]. This happens due to the urgent need of lowering their high surface tension and is therefore a much stronger force than established by hydrophilic surfaces. Balafilcon A is simultaneously anionic and hydrophobic, making this lens virtually more prone to biocide uptake. In addition to this, balafilcon A exhibits large pores [33] that should favour the biocide uptake by the lens matrix. The material presenting greater disinfection scores was the conventional hydrogel polymacon. In fact this material is hydrophilic, nonionic characteristics that limited the entrance of the biocide onto their matrix. Polymacon has given uniformly better log unit reduction than the materials now frequently used in the clinical practice and their contribution as a test material in ISO standards could be considered.

The log reduction on lenses treated with Opti-Free® was generally more accentuated than with PHBM-based MPS (Table 4). Similar findings were reported by Rosenthal et al. The authors found that the number of survivors on lenses treated with Opti-Free® was smaller in comparison with other MPS [23]. The reasons behind its greater disinfection efficacy are multiple. The first appears to be related with its little propensity to be uptaken either by the CL or the lens case. Opti-Free® contains a large molecular size biocide known as PQ-1, whereas Renu® and Complete® contain PHMB which is a smaller molecule. PQ-1 due to its size is less probable to be sequestered by the CL matrix or lens cases, therefore, maintaining their antimicrobial activity. So, the enhanced performance of Opti-Free® might be ascribed to low levels of PQ-1 adsorption onto CLs. A second factor that might explain the superior performance of Opti-Free® is their superior biocide concentration, a key factor in disinfection [34]. Opti-Free® contains 0.001% of PQ-1, while Renu® and Complete® MoisturePlus™ contain 0.0001% of PHMB. Finally, the greater disinfection efficacy of Opti-Free® may be attributed to the presence of 2 biocides, PQ-1 and MAPD, rather than one. MAPD is a biocide composed by a fatty acid coupled to a cationic group with demonstrated effectiveness against fungi, and trophozoide and cystic form of *Acanthamoeba* [35]. Although being an effective antifungal it has also an antibacterial action [36] and may thus contribute for the best disinfection efficacy found for Opti-Free®.

A convenient use of MPS helps to control the microbial load and should reduce the chances of developing any of these ocular events. However the present study suggests that the MPS formulation as well as the CL material affects the disinfection efficacy of a contaminated CL, so special care must be taken when combining a certain CL material with a MPS.

Table 4

Number of survivors on contact lenses after treatment with Opti-Free®.

CL material	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
Balafilcon A	4.33 (1.15)	22.60 (13.83)	1.60 (1.14)
Lotrafilcon A	3.33 (0.876)	3.33 (1.77)	5.50 (2.14)
Polymacon	0.00 (0.00)	3.83 (2.43)	6.33 (2.05)
Etafilcon A	1.00 (0.57)	3.60 (1.95)	3.2 (1.96)

Acknowledgements

The authors fully acknowledge the financial support of the Portuguese Foundation for Science and Technology (FCT) by the grant SFRH/BD/19679/2004.

References

- [1] Szczotka-Flynn LB, Bajaksouzian A, Jacobs MR, Rimm A. Risk factors for contact lens bacterial contamination during wear. *Optom Vis Sci* 2009;86:1216–26.
- [2] Mély RG. Sterile cornea infiltrates related to soft contact lens wear. In: CLAO annual meeting. 2001.
- [3] Cheng KH, Leung SL, Hoekman HW, Beekhuis WH, Mulder PG, Geerards AJ, et al. Incidence of contact lens-associated microbial keratitis and their relative morbidity. *Lancet* 1999;354:181–5.
- [4] Lakki C, Fleiszig SMJ. Resistance of *Pseudomonas aeruginosa* isolates to hydrogel contact lens disinfection correlates with cytotoxic activity. *J Clin Microbiol* 2001;39:1477–86.
- [5] Mondino BJ, Weissman BA, Farb MD, Pettit PH. Corneal ulcer associated with daily wear and extended wear contact lenses. *Arch Ophthalmol* 1986;98:1767–70.
- [6] Morgan PB, Efron N, Hill EA, Raynor MK, Whiting MA, Tullo AB. Incidence of keratitis of varying severity among contact lens wearers. *Br J Ophthalmol* 2005;89:430–6.
- [7] Nayak N, Nag TC, Satpathy G, Ray SB. Ultrastructural analysis of slime positive and slime negative *Staphylococcus epidermidis* isolates in infection keratitis. *Indian J Med Res* 2007;125:767–71.
- [8] Schornack MM, Peterson D. *Staphylococcus aureus* ulcer associated with continuous wear of silicone hydrogel contact lenses. *Eye Contact Lens* 2006;32(2):72–4.
- [9] Willcox MDP, Holden BA. Contact lens related corneal infections. *Biosci Rep* 2001;21:445–61.
- [10] Zhu H, Ding A, Bandara M, Willcox MD, Stapleton F. Broad spectrum of antibacterial activity of a new multipurpose disinfecting solution. *Eye Contact Lens* 2007;33:278–83.
- [11] Willcox MDP, Harmis N, Cowell BA, Williams T, Holden BA. Bacterial interactions with contact lens; effects of lens material, lens wear and microbial physiology. *Biomaterials* 2001;22:3235–47.
- [12] Carnt NA, Evans V, Naduvilath TJ, Willcox MDP, Papas EB, Frick KD, et al. Contact lens-related adverse events and the silicone hydrogel lenses and daily wear care systems used. *Arch Ophthalmol* 2009;127:1616–23.
- [13] Jones L, MacDougall N, Sorbara LG. Asymptomatic corneal staining associated with the use of balafilcon silicon-hydrogel contact lenses disinfected with a polyaminopropyl biguanide-preserved care regimen. *Optom Vis Sci* 2002;79:753–61.
- [14] Tighe B. Soft lens materials. In: Efron N, editor. *Contact lens practice*. Oxford: Butterworth-Heinemann; 2002. p. 71–84.
- [15] Guryča V, Hobzová R, Pěšáková M. Surface morphology of contact lenses probed with microscopy techniques. *Contact Lens Anterior Eye* 2007;30: 215–22.
- [16] Tighe B. Silicone hydrogels: structure, properties and behaviour. In: Sweeney D, editor. *Silicone hydrogels: continuous wear contact lenses*. Oxford: Butterworth-Heinemann; 2004. p. 1–27.
- [17] Ophthalmic optics - Contact lens care products - Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses. International Organization for Standardization: ISO/CD 14729:Ophthalmic Optics 2001;1–21.
- [18] Hume EB, Flanagan J, Masoudi S, Zhu H, Cole N, Willcox MD. Soft contact lens disinfection solution efficacy: clinical *Fusarium* isolates vs ATCC 36031. *Optom Vis Sci* 2009;86(5):415–9.
- [19] Scheuer C, Zhao F, Erb T, Orsborn G. Multipurpose solutions and rates of biocidal efficacy. *Eye Contact Lens* 2009;35(2):88–91.
- [20] Russell AD. Biocide and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. *Infect Dis* 2003;3:794–803.
- [21] Russell AD. Bacterial adaptation and resistance to antiseptics, disinfectants and preservatives is not a new phenomenon. *J Hospital Infect* 2004;57(2):97–104.
- [22] Santos L, Rodrigues D, Lira M, Real Oliveira MECD, Oliveira R, Vilar EY-P, et al. The influence of lens material and lens wear on the removal and viability of *Staphylococcus epidermidis*. *Contact Lens Anterior Eye* 2008;31:126–30.
- [23] Rosenthal R, Dassanayake NL, Schlitzer RL, Schlech BA, Meadows DL, Stone RP. Biocide uptake in contact lenses and loss of fungicidal activity during storage of contact lenses. *Eye Contact Lenses* 2006;32:262–6.
- [24] Publication passport ISO 14729, 2001. StrainInfo^{BETA} <http://www.straininfo.net/publications/109751>.
- [25] Cerca N, Pier GB, Oliveira R, Azeredo J. Comparative evaluation of coagulase-negative staphylococci (CoNS) adherence to acrylic by static method and a parallel-plate flow dynamic method. *Res Microbiol* 2004;155:755–60.
- [26] Cerca N, Pier GB, Vilanova M, Oliveira R, Azeredo J. Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of *Staphylococcus epidermidis*. *Res Microbiol* 2005;156:506–14.
- [27] Sentell KB, Beaulieu E. Comparison of preservative uptake and release profiles of PHMB from soft contact lenses care products by silicone hydrogel contact lenses. *Invest Ophthalmol Vis Sci* 2004;45:1573. E-Abstract.
- [28] Dannelly HK, Waworuntu RV. Effectiveness of contact lens disinfectants after lens storage. *Eye Contact Lens* 2004;30:163–5.
- [29] Andrasko G, Ryan K. Corneal stinging and comfort observed with traditional and silicone hydrogel lenses and multipurpose solution combinations. *Optometry* 2008;79:444–54.
- [30] Powell CH, Lally JM, Hoong LD, Huth SW. Lipophilic versus hydrodynamic modes of uptake and release by contact lenses of active entities used in multipurpose solutions. *Contact Lens Anterior Eye* 2010;33:9–18.
- [31] Holmberg K, Jönsson B, Kronberg B, Lindman B. Surfactants and polymers in aqueous solution. 2nd edition John Wiley & Sons; 2002 [chapter 17, p. 357–87].
- [32] Kevelam J, van Breemen JFL, Blokzijl W, Engberts JBFN. Polymer-surfactant interactions studied by titration calorimetry: influence of polymer hydrophobicity, electrostatic forces, and surfactant aggregational state. *Langmuir* 1996;12:4709–17.
- [33] López-Alemán A, Compañ V, Refojo MF. Porous structure of PurevisionTM, versus Focus[®] Night&DayTM and conventional hydrogel contact lenses. *J Biomed Mater Res Part B Appl Biomater* 2002;63:319–25.
- [34] McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action and resistance. *Clin Microbiol Rev* 1999;12:147–79.
- [35] Gavin J, Button NF, Watson Craik IA, Logan NA. Observation of soft contact lenses disinfection with fluorescent metabolic stains. *Appl Environ Microbiol* 2000;66:874–5.
- [36] Codling CE, Maillard JY, Russell AD. Aspects of antimicrobial mechanisms of action of a polyquaternium and an aminodiamine. *J Antimicrob Chemother* 2003;51:1153–8.