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Evaluating the cytotoxicity of contact lens multi-purpose solutions in an *in vitro* lens system

O Matthew Oriowo

Department of Optometry, College of Applied Medical Sciences, King Saud University, PO Box 10219, Riyadh, 11433 Saudi Arabia

<matoriowo@yahoo.com>

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Abstract

Purpose: To investigate the relative cytotoxic effects of contact lens multipurpose solutions on cultured crystalline lenses.
Methods: A comparison of the fluorescence emission levels of cultured bovine lenses as affected by three hour experimental exposure to three contact lens multipurpose solutions (COMPLETE MoisturePlus, AMO; OPTI-FREE Express, Alcon; and ReNu MultiPlus, Bausch & Lomb) was carried out. The pre- and post-exposure fluorescence levels of the lenses were obtained and values were compared to baseline and control measurements.
Results: The solutions yielded varying degrees of cytotoxicity, demonstrating significant ($p < 0.01$) reversible reduction of cellular viability levels of the cultured crystalline lenses as revealed by the degree of fluorescence emissions in the following order (OPTI-FREE Express > ReNu MultiPlus > COMPLETE MoisturePlus multi-purpose solutions).
Conclusions: The results show that OPTI-FREE Express and ReNu MultiPlus solutions exhibited more cytotoxic effect compared to COMPLETE MoisturePlus solution. The findings support reports from previous clinical and laboratory studies. These results suggest that the *in vitro* approach herein presented would be a valuable system for relatively inexpensive and repeatable laboratory investigations of the possible ocular surface reactions of ophthalmic solutions, cosmetics and pharmaceuticals at pre- and during commercial phases.

Keywords: Contact lens multipurpose solutions; ocular lens; cell viability; cytotoxicity; Alamar-Blue; biochemical assay; fluorescence.

Introduction

The need for contact lens practitioners to have a basic understanding of the components and the temporary ocular surface reactions from using contact lens care solutions cannot be overemphasised. This will arm the practitioner with useful knowledge with respect to what to tell patients regarding the transient ocular irritation that is possible from the use of multipurpose contact lens solutions. Currently, the most common products for disinfecting contact lenses are

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multi-purpose solutions (MPS) that can be used to clean, disinfect and wet contact lenses. Such a solution must be potent enough to kill microbial pathogens that may be harboured on the contact lenses yet must also be particularly gentle to the eye. A recent study found that contact lens wear failure was related to the product or practitioner factor rather than patient-specific problems[1], implying that practitioners should possess adequate knowledge and be able to advise patients on the relative ocular surface reactions to different contact lens solutions.

It is a common view among eye care providers that efficacy against pathogenic microbes cannot be compromised in an attempt to produce an irritancy-free contact lens care solution since contact lens wear and contaminated contact lens solutions are the main causes of microbial keratitis[2] . For example clinical and laboratory studies have observed that the COMPLETE® multi-purpose solution has minimal toxicity compared to OPTI-FREE and ReNu solutions[3, 4]. The fact that a solution is more comfortable, that is, has minimal cytotoxic or sensitivity effects does not guarantee full efficacy against opportunistic pathogens. For instance, recent results from two independent epidemiologic studies[5, 6], found that ~50% - 55% of *Acanthamoeba keratitis* (AK) cases used COMPLETE MoisturePlus multi-purpose solution, resulting in a more than 15-fold increase in the risk of AK with COMPLETE MoisturePlus solution use. This led the manufacturer, Advanced Medical Optics (AMO) to voluntarily recall the COMPLETE MoisturePlus multi-purpose solution[7].

Contact lens multipurpose solutions (MPS) have different compositions as indicated in Table 1.

Table 1. Composition of the various multipurpose solutions.

Solutions	Preservatives	Surfactant/cleaner	Buffer	Other components (e.g. Electrolyte)
*COMPLETE Moisture Plus™	0.0001% polyhexamethylene biguanide (PHMB)	Poloxamer 237	Sodium phosphate	Potasium chloride, NaCl, EDTA (0.01%), Taurine, Propylene Glycol.
ReNu MultiPlus	0.0001% polyaminopropyl biguanide (PAPB)	Poloxamine, hydroxyalkyl phosphonate	Sodium borate/boric acid	EDTA (0.1%), NaCl
OPTI-FREE Express	0.001% polidronium chloride, 0.0005% myristamidopropyl dimethylamine (MAPD)	AMP-95, Tetronic 1304	Sodium citrate/Boric acid	Sodium chloride (NaCl), sorbital, Edetate disodium (EDTA, 0.5%)

*The COMPLETE® Moisture Plus™ multi-purpose solution has lubricant/conditioner called Hydroxypropyl methyl cellulose (HPMC) in its formulation.

Manufacturers indicate that the preservatives (that is, antimicrobial agents) and other additives in the solutions have high molecular weight materials which should not normally penetrate the contact lens matrix. Hence preventing any build up to toxic levels[8, 10]. It has been a concern that MPS preserved with even low percentages of antimicrobial agents could cause ocular surface irritation. Within the last twenty years, a number of no-rub MPS have been introduced[4, 10-13]. MPSs are convenient and simple to use, but may present a compromise of the cleaning and disinfecting functions[13-15]. The reason for non-compliance among contact lens wearers is multifactorial and well documented in the literature[16-20]. The notion that ocular surface sensitivity to MPS may contribute to non-compliance and contact lens dropout is still controversial[3, 21]. The manufacturers' efforts to provide simplified care systems may lead practitioners and patients into a false sense of security and the use of disposable or frequent replacement lenses may cause them to place less emphasis on the cleaning of lenses[22]. The incidence and morbidity of contact lens-related mi-

crobial keratitis have shown little change compared with reports in the late 1980's[6, 23, 24]. Despite changes or claimed improvements to contact lens care solutions, microbial keratitis is still a concern in contact lens wear today, particularly in extended contact lens wear[24, 25]. Thus, contact lens care solutions must be efficacious against any pathogenic expressions by the microbial flora in the ocular surface.

Since contact lens MPS(s) have varying levels of antimicrobial, cleaning and lubricating activities[26-29], they will inevitably present some variations in their level of ocular surface sensitivity. The OPTI-FREE Express MPS has been found to show the highest antibacterial activity against *Pseudomonas aeruginosa* compared to ReNu and COMPLETE solutions[29], which should suggest more ocular surface cytotoxic or cytosensitive effect. However, a recent study reported that ReNu MultiPlus showed a more significant adverse ocular surface sensitivity effect compared to OPTI-FREE and COMPLETE solutions[3]. Hence, the objective of the present study was to investigate the relative cytotoxicity of three commonly used contact lens multipurpose solutions (COMPLETE moistureplus, OPTI-FREE Express and ReNu MultiPlus) to bovine lenses, using an *in vitro* approach. This *in vitro* approach employing the Alamar Blue™ biochemical assay with cultured ocular lens was recently introduced[30-32]. A repeatable *in vitro* approach to perform comparative sensitivity evaluations among contact lens solutions would be essential, particularly when considering cost effectiveness, the need for rapid screening information and to avoid the traditional large variation that occurs with *in vivo* studies such as the Draize test using rabbits[16, 17, 19, 33-36]. The Alamar Blue™ bioassay method utilizes the fluorescence emission levels of cultured whole crystalline lens tissue, as measured with a fluorescence multi-plate reader. The Alamar Blue™ assay model has shown consistent repeatability in its ability to detect subtle cytotoxic changes in ocular lens culture and human conjunctival cell lines[4, 30-32]. The use of bovine crystalline lens is relevant practically and experimentally. The crystalline lens was chosen as an ocular tissue model for studying ocular tissue irritancy because; (1) The epithelium of the cornea and the lens have the same embryologic origin and are physiologically similar. (2) The main function of both structures is to focus an image on the retina. (3) The structural adaptations of both tissues are designed to minimize light scatter, and (4) both the lens and cornea are avascular[32, 36].

Methods

Multipurpose contact lens solutions

Fourteen bottles of each of the three multipurpose solutions: ReNu MultiPlus (Bausch & Lomb, Rochester, NY, USA); OPTI-FREE Express (Alcon Laboratories, Fort Worth, TX, USA); and COMPLETE moistureplus (Advanced Medical Optics, Dublin, Ireland) were randomly purchased from commercial retail pharmacy stores as encountered by the public. The composition of the solutions is as shown in Table 1.

Crystalline Lens culture

All culture ingredients were purchased from the Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated. Whole crystalline lenses were excised under aseptic conditions from abattoir-obtained bovine eyes and placed in a custom 25-ml two-chambered container. The bovine eyes, from two year old cattle stock were obtained within 1-2 hour *post-mortem*, and held at room temperature until the dissection of the lenses, which occurred within 2 to 5 hour *post-mortem*. To isolate the lens, the posterior portion of the eyeball was aseptically dissected, the suspensory ligaments of the lens were cut, and the adhering vitreous removed. The lenses were immersed in M199 culture medium with 3% fetal bovine serum and 1% antibiotics (penicillin/streptomycin 100 units per ml) with sodium bicarbonate and HEPES as buffers for pH stability. The cultured lenses were incubated at 37°C in an air, 5% CO2 atmosphere when not undergoing experimental measurements.

Exposure of crystalline lenses to contact lens multipurpose (MPS) solutions

The dissected lenses were kept in culture medium for 48 hours to allow for adaptation of the lenses to the medium. After the 48 hours of placing the lenses in culture medium, 40 out of 55 excised lenses were randomly allotted to treatment (utilizing 10 lenses per each experimental solution) group, and 10 lenses for the control group. The same set of 10 lenses was utilized as control for the three experimental solution groups. Fifteen lenses were not included in the study due to physical damage during dissection. The 30

lenses selected (10 for each MPS solution treatment) for exposure were completely submerged in the three experimental solutions, respectively, for three hours at room temperature under the biological flow hood. The experimental exposure solutions comprised undiluted contact lens MPS, poured directly from the dispensing bottles into the culture chamber, each containing one lens. Untreated control lenses remained in the culture medium for three hours at room temperature under the flow hood. All procedures were done under sterile conditions utilizing a biohazard approved biological flow hood. After the three hour exposure period to MPS, the treated lenses were rinsed and the MPS replaced with fresh culture medium, and all lenses were returned to the incubator.

Biochemical assay and fluorescence measurements

Baseline fluorescence measurements were obtained for control and treated lenses prior to exposure, and at the 6, 12, 24, 48, and 96 hour time intervals post the 3-hour experimental exposure to contact lens MPS. Following each measurement session, all lenses were rinsed and returned to fresh culture medium. The biochemical assay system consisted of the Alamar Blue™ assay, 12 multiwell plates, and a fluorescence plate reader. The system quantifies and records the fluorescence intensity levels of cultured ocular lenses. The fluorescence measurement is based on the principle that as radiant light wavelength is absorbed by a substance (in this case, the crystalline lens immersed in Alamar Blue™ assay) with the excitation wavelength of 530 nm, a certain amount of fluorescence emission will result at a longer wavelength (in this case at 590 nm). The Alamar Blue™ dye (obtained from MEDICORP Inc., Montreal, Canada) is used to quantify the viability level of living cells *in vitro*[37]. It incorporates resazurin and resorufin as a fluorometric-colorimetric oxidation-reduction (Redox) indicator that fluoresces and changes colour in response to reduction resulting from cell metabolism[37, 38]. It has been reported that serum interferes with Alamar Blue™ fluorescence readings by inducing reduction of the dye[39], therefore it was ensured that the experimental assay medium was serum free.

The Alamar Blue™ dye was diluted into the culture medium (modified M199) without serum, to 8% (v/v). The assay solution was prepared immediately before use at each measurement session using a 100 μl Eppendorf® "Tip-Ejector" microlitre pipette. Freshly prepared assay solution before each use at every measurement time point was to avoid possible precipitation of the assay dye. Both the treated and control lenses were transferred into sterile 12-well flat bottom tissue culture plates (Costar, Cambridge, MA, USA) with one lens per well. The culture medium containing serum was carefully aspirated, and the lenses rinsed twice with 6-ml of experimental medium with no serum. Then 3.8-ml of the assay solution was added to each well containing a lens, using a sterile 250- μl adjustable pipette tip and an Eppendorf repeater pipette. A prior pilot study by the present investigator showed a two hour incubation period to be optimum for the assay to diffuse into the lenses for fluorescence measurement. Therefore at each measurement session, both control and experimental lenses were incubated for two hours in the assay solution, after which the fluorescence measurements were performed with a CytoFluor™ II fluorescence multiwell plate reader (PerSeptive Biosystems Inc., Framingham, MA, USA). Thirty minutes prior to performing the fluorescence measurements, the excitation / emission wavelength settings on the CytoFluor™ plate reader were adjusted to 530/590 nm with the sensitivity gain set at 50, and temperature at 37ºC. The plate reader probe was set to scan 10 different positions in each lens. Thus, an average of 10 readings was obtained for every lens. Therefore, each fluorescence level data represent an average of 10 readings per scan for each lens at least six times through the study duration, amounting to a total of 2400 quantitative lens fluorescence intensity measures.

Statistical analysis

Results were expressed as the mean ± the standard deviation (S.D) of the mean. The data were analysed using the paired *t*-test and repeated-measures analysis of variance (ANOVA). Repeated measures ANOVA was used to compare the control group with the treated groups of lenses across all six measurement time periods. A repeated measures ANOVA found a significantly interaction between time and group ($p < 0.001$). For within group analysis, the baseline (that is, pre-exposure) and follow up post-exposure fluorescence readings of lenses in the same group at the predetermined intervals were compared. The data were also compared between groups at each time point. The Dunnett multiple statistical test was used for within group comparisons (comparing baseline to

subsequent measurements in the same group), and the Tukey-Kramer multiple comparisons test for between group comparison at baseline and the respective follow-up time points. Any probability *p* value less than 0.01 was considered significant. The extent of cytotoxicity is judged by each *p* value which indicates the degree of significant effects as follows: *p* values equal or less than 0.01(significant); ≤ 0.001 > 0.0001 (highly significant); and ≤ 0.0001 > 0.00001 (extremely significant). The total number of times each solution exhibited minimal to extremely significant effect will be used to determine and discuss the relative rank order of cytotoxicity among the solutions.

Results

The results for the different relative cytotoxic effects of the solutions are shown in Tables 2 to 4 as the mean (±S.D). At 6 hours, lenses treated with OPTI-FREE and ReNu (Tables 2 and 3) demonstrated significant cytosensitive effects with *p* values of 0.001 and 0.01, respectively. The control lenses (*n* = 10), and lenses treated with COMPLETE solution (*n* = 10) did not show significant changes in fluorescence profiles throughout the duration of the experiment (Table 4). The OPTI-FREE treated lenses exhibited a significant recovery at 12 hr, but with a rebounce at 24 hr (*p* = 0.00001) and eventually back to recovery by 96 hr. The fluorescence levels of ReNu treated lenses did not show a recovery at 12 hr (*p* = 0.012), but exhibited gradual recovery beginning from 24 hr to baseline and control levels by the end of the 96 hr study (Table 3).

In order to rank the order of cytotoxicity among the three solutions, it was considered that the level of significance at each time point would indicate the degree of cytotoxic effect of each solution. Therefore, judging from the *p* values for each solution at different intervals as shown in Tables 2 - 4, the descending rank order of the cytotoxic effect is as follows: OPTI-FREE > ReNu > COMPLETE solutions, with OPTI-FREE showing the most cytotoxicity effect.

Table 2. Descriptive statistics of the fluorescence data for lenses exposed to OPTI-FREE multipurpose contact lens solution (*n* = 10 for exposed lenses, and *n* = 10 for control lenses).

Measurement time (hr)	Fluorescence level (mean ± S.D.)		*p* values (within group) Contrast to baseline		*p* values (Between groups) Exposed versus Control
	Exposed	Control	Exposed	Control	
Baseline	34656 ± 2855	34835 ± 3811			0.907
6	28007 ± 3866	37042 ± 3379	0.001	0.10	0.00003
12	33820 ± 3287	35692 ± 4192	0.574	0.62	0.281
24	27751 ± 3132	35431 ± 2360	0.00001	0.71	0.00002
48	27007 ± 2819	33647 ± 2834	0.00001	0.13	0.00005
96	32952 ± 3651	35725 ± 4585	0.245	0.60	0.153

Note: Baseline readings were taken before exposing lenses to MPS. The same set of control lenses were utilised for OPTI-FREE, Complete, and ReNu solution exposures. Values are presented as arbitrary fluorescent units. Grading the level of cytotoxicity at different time points: *p* = 0.01 (minimal), 0.001 (very adverse), < 0.0001 (extremely adverse).

Table 3. Descriptive statistics of the fluorescence data for lenses exposed to ReNu multipurpose contact lens solution (*n* = 10 for exposed lenses, and *n* = 10 for control lenses).

Measurement time (hr)	Fluorescence level (mean ± S.D.)		*p* values (within group) Contrast to baseline		*p* values (Between groups) Exposed versus Control
	Exposed	Control	Exposed	Control	
Baseline	34720 ± 3313	34835 ± 3811			0.944
6	28392 ± 5295	37042 ± 3379	0.005	0.10	0.001
12	28141 ± 5745	35692 ± 4192	0.012	0.62	0.002
24	31161 ± 2887	35431 ± 2360	0.016	0.71	0.002
48	35088 ± 2973	33647 ± 2834	0.820	0.13	0.282
96	35157 ± 4166	35725 ± 4585	0.585	0.60	0.775

Table 4. Descriptive statistics of the fluorescence data for lenses exposed to COMPLETE multipurpose contact lens solution (n = 10 for exposed lenses, and n = 10 for control lenses).

Measurement time (hr)	Fluorescence level (mean ± S.D.)		p values (within group) Contrast to baseline		p values (Between groups) Exposed versus Control
	Exposed	Control	Exposed	Control	
Baseline	35599 ± 3881	34835 ± 3811			0.66
6	36381 ± 2389	37042 ± 3379	0.55	0.10	0.62
12	35537 ± 2763	35692 ± 4192	0.96	0.62	0.92
24	34470 ± 1903	35431 ± 2360	0.42	0.71	0.33
48	35205 ± 1727	33647 ± 2834	0.70	0.13	0.16
96	35744 ± 3658	35725 ± 4585	0.91	0.60	0.99

Discussion

The fact that the last decade has witnessed contact lens wearers changing to disposable and frequent replacement soft lenses with multipurpose solutions requires the contact lens practitioner to have informed knowledge on the efficacy and relative irritancy of multipurpose contact lens care regimens[3, 10, 25]. Contact lens multipurpose solutions contain quaternary ammoniums or polymers of hexamethylene biguanide (PHMB) as active preservative agents. Preservatives are widely used in agricultural and food chemistry to keep food fresh, and in the cosmetic industry to avoid spoilage or chemical changes by microbes such as bacteria and fungi. Preservatives are also commonly used as sanitizers for baby wipes, pool and spa, and as disinfection products in medical preparations such as eye drops or contact lens solutions[4].

Multipurpose solutions are classified as medical devices (class 2b) and can impregnate the contact lens during the soaking time and insertion on ocular surface. Chemicals including preservatives contained in contact lens solutions could initiate ocular surface cytotoxic reactions or contact lens intolerance. The findings of the present study show that the three contact lens MPSs induced varying levels of reversible lens cytotoxic reactions, with OPTI-FREE Express No Rub® solution showing the most effect. Similar observations on cultured human conjunctival cell lines showed that OPTI-FREE Express solution was significantly more toxic than ReNu MultiPlus No Rub® and COMPLETE moistureplus™ solution, respectively[4]. In contrast, an *in vivo* observation from a recent clinical study found that ReNu MultiPlus produced the most adverse ocular surface effect compared to OPTI-FREE and COMPLETE solutions[3].

From the findings in the present study it might be assumed that the COMPLETE moistureplus™ MPS would have relatively little sensitivity effect compared to OPTI-FREE or ReNu. However, there is a caveat in the assumption in that the present study is an exploratory *in vitro* investigation as opposed to *in vivo* experiment in which actual ocular surface irritancy effects can be directly obtained. A future investigation is required to conduct a parallel study of *in vitro* and *in vivo* evaluation of sensitivity effects of contact lens MPSs, and study the relationship between ocular lens cytotoxicity findings and actual ocular surface sensitivity effects. One possible explanation for the difference in the MPS cytotoxic effects is that OPTI-FREE solution has a completely different antimicrobial agent (0.0005% myristamidopropyl dimethylamine (MAPD), while ReNu and COMPLETE moistureplus MPSs are both 0.0001% of polyhexamethylene biguanide (PHMB)-preservative based solution systems. The antimicrobial action of MAPD is not fully known but has been proposed as similar to the action of PHMB and chlorhexidine which causes cytoplasmic membrane damage leading to loss of essential cellular components following binding to the cell wall[40]. Also, as shown in Table 1, the COMPLETE solution has hydroxypropyl methylcellulose (HPMC) as lubricant in its formulations compared to ReNu and OPTI-FREE solutions. The findings in the present study indicate that the chemical variations which exist between the solutions would yield differential cytotoxic reactions. However, the results show that there will be

recovery from such irritancy or cytotoxic effect from the solutions.

In the field of toxicology, the Draize[41] test has been the standard *in vivo* measure of ocular toxicity for over fifty years. It is based on observations of irritation and injury to the cornea, conjunctiva, and iris after the application of test chemicals to the eyes of live rabbits[33, 34]. However, because of poor sensitivity and repeatability and ethical concerns about the suffering of live animals, there is an increasing need for more *in vitro* alternatives[33, 34, 36], and researchers have continued to develop more *in vitro* approaches for determining ocular toxicity[30, 36, 42]. Crystalline lens culture has increasingly been used for *in vitro* alternative methods in ocular toxicology[30, 32, 36, 42]. Unlike the cornea, the ocular lens can be cultured as an intact organ for long periods as it can retain its physiological integrity during the culture period, particularly with its repair mechanisms preserved. As earlier mentioned the crystalline lens has a number of similarities to the cornea to support its use as a model for corneal irritancy testing. It is an avascular tissue and its principal function is to transmit light to the retina. Both the lens and cornea have structural and physiological adaptations to refract light.

According to the findings in the present study, there is no indication that any of these solutions would result in permanent adverse cytotoxic damage to the ocular surface tissue with clinical or patient care use. Concerning the trade-off between relative efficacy and comfort, the findings of relative cytotoxic effect between the three solutions as demonstrated in the present study appear to agree with the findings of Leung *et al*.[29], who found that OPTI-FREE Express showed the highest antimicrobial activity against *Pseudomonas aeruginosa* compared to ReNu, COMPLETE and Solo-care solutions at 4°C, 25°C and 30°C, however this is not the focus of the present study. An ideal contact lens MPS would have both low cytotoxic effect and high antimicrobial efficacy. Efficacy will always be of paramount importance in contact lens multipurpose solutions because it has been found that even a standard contact lens care hygiene regime does not seem to be sufficient in preventing the development of corneal infection and ulcers in contact lens (particularly in conventional and frequent replacement daily wear soft contact lenses) wearers[25, 43]. In terms of *in vitro* methodology, the Alamar Blue™ biochemical assay has been used in many fields, especially in pharmacology to screen for agents toxic to mammalian cells[44]. The results in the present study agree with the previous clinical/laboratory findings[3, 45], and those of Pharm and Huff [46] who used the Alamar Blue™ assay to study cytotoxic effect of contact lens solutions on bovine corneal epithelial cultures.

In conclusion, these results confirm that OPTI-FREE is more cytotoxic compared to ReNu and COMPLETE contact lens multipurpose solutions. The *in vitro* system herein presented offers a quick and quantitative *in vitro* assessment of the efficacy and potential irritability of contact lens solutions. As well, it would be a valuable system for relatively inexpensive and repeatable laboratory investigations of the possible ocular surface reactions of ophthalmic solutions, cosmetics and pharmaceuticals at pre- and during commercial phases.

Declaration:

The author has no proprietary or commercial interest in the products named in this article.

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