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INFLUENCES OF HYDROGEL CONTACT LENS CARE SOLUTIONS ON CORNEAL EPITHELIAL WOUND HEALING

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We studied the influences of hydrogel contact lens care solutions on corneal epithelial wound healing in vitro. The corneal epithelial wounds, 1.5mm in diameter, 70μm in depth were created on the pig's eyeballs by the excimer laser. The hydrogen peroxide systems including AOSEPT, Oxysept, Contopharma-peroxide-system were neutralized first, then applied three times on the epithelial wounds. The neutralizing procedures included the agents, duration, doses and containers following the instructions accompanied in the package of the solutions. The multipurpose care solutions including Opti-free, Hexidin, Bausch & Lomb Sensitive Eyes were applied directly as well. The healing scores were ranked 24 hours later with the fluorescein stain. There were no statistical differences between the healing scores of each experiment group and control group performed with Mann-Whitney rank-sum test. None of the lens care solutions retarded the corneal epithelial wound healing when compared with the BSS. The contact lenses treated with above solutions may be safely applied on eye without rinsing.

Key words: hydrogel contact lens, corneal epithelium, lens care solution

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A development of contact lens solutions should consider three main issues: efficacy, safety, and convenience. High concentration of antimicrobial agents, though efficient for sterilization, tends to be toxic especially with hydrogel lenses, because they have higher potential to bind and hold the toxic compounds in and on the lenses. The adverse effects on eye have been reported either with chemical disinfecting solution or hydrogen peroxide[1,2]. The antimicrobial agent must be neutralized or rinsed off before the lens can be applied on eye. Since they are more convenient, multipurpose solutions have become widely accepted by the lens wearers. These agents do not require the final rinse step, and large amounts of the solutions will be brought to the eye surface through the contact lens insertion. The hydrogen peroxide systems are neutralized by different methods. The misuse or decay of neutralization agents may induce corneal damage by the residual hydrogen peroxide. The toxicity of hydrogel lens care solutions should be carefully evaluated to prevent potential damage to the ocular surface. We have developed an in vitro corneal epithelial wound healing model to survey the toxicity of ocular agents. In this study, we utilized this model to evaluate the toxicity of the hydrogel lens care solutions.

MATERIALS AND METHODS

All of the hydrogel contact lens care solutions were purchased from the market. The hydrogen peroxide systems including AOSEPT (CIBA vision), Oxysept comfort (Allergan), Contopharma-peroxide-system (Contopharma) were neutralized before application on the epithelial wounds. The neutralizing procedures in-

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cluding the neutralizing agents, duration, doses and the containers all followed the instructions accompanying the solutions. The multipurpose solutions included Opti-free (Alcon), Hexidin (Pilkington Barnes Hind) and B&L Sensitive Eyes (Bausch & Lomb). All of the above solutions stated that the contact lenses stored in such a kind of solutions could be placed on eyes without rinsing. This group of solutions were aspirated from the containers and applied directly to the corneal epithelial wounds.

Pig's eyeballs were collected from the local slaughterhouse and kept on ice during the transportation and before the experiment. The adnexa tissues and conjunctiva were removed first. Then the eyeballs were immersed in 3% polyvinyl pyrrolidine iodine for 1 minute and 0.1% gentamycin for 3 minutes for disinfection. The excimer laser (Schwind keratome) at 193nm created a central corneal epithelial wound through a phototherapeutic procedure under a fluence of 270 mJ/cm^2. The wound was 1.5mm in diameter and 70 μ m in depth. Six eyeballs were tested for each experimental group and eight eyeballs for the control group.

After treatment, the eyeballs were placed on a special design bench with holes, which could keep the corneas in an upright position. The benches were kept in an incubator under the criteria of 37°C, 5% CO_2 and 100% humidity. The eyeball was perfused with TC-199 medium through an intravenous infusion set that connected the medium bottle outside the incubator on one end and a 25 gauge needle punctured into the vitreous cavity at the equator of the eyeball on the other end.

Right after the eyeballs were placed into the incubator, TC-199 media were applied on the corneas to rinse the surface. One, three and five hours later after setup, three drops of the testing solutions or balanced salt solution (BSS) as control were applied on the epithelial wounds. Twenty-four hours later, the wounds were stained with fluorescin and evaluated under a blue light. The sizes of stained epithelial defects were ranked with a scoring system from 3 to 0, according to the healing states from poor to good. The details of the experiment setup and the evaluation criteria of the scoring system have been described in our previous reports[3,4]. Statistical analysis to verify the differences between experiment and control groups was performed using the Mann-Whitney rank-sum test. Statistical significance was accepted at $p \leqq 0.05$.

RESULTS

All of the solutions whether the hydrogen peroxide or the multipurpose systems, revealed no retarding effect on the corneal epithelial wound healing. Statistical differences were not significant between each experiment group and the control group. The healing scores of each group and the statistical p value comparing experiment and control group are shown in Table 1.

DISCUSSION

Hydrogen peroxide has been used as a disinfectant for a century. Isen first introduced it as a means of contact lens disinfection[5]. The early hydrogen peroxide system possessed no suitable agent for neutralization; multiple saline rinse was the way to reduce the concentration. It was regarded as cumbersome and potentially dangerous, and was not well accepted. Since too many contact lens wearers were allergic to thimerosal and chlorhexidine, which were the main components

Table 1. Healing scores of the experiment and control groups

Solutions		Healing score of each cornea								p values*
AO SEPT	n=6	0	0	1	0	1	1			0.541
Oxysept	n=6	0	0	2	2	0	1			0.363
Contopharma	n=6	1	1	0	0	1	2			0.217
Opti-free	n=6	2	0	1	1	1	1			0.089
Hexidin	n=6	2	0	0	0	2	0			0.684
B&L	n=6	1	1	0	0	0	1			0.541
Control	n=8	0	0	0	0	0	2	0	1	

*Statistical analysis for the differences between experiment and control groups. Mann-Whitney rank-sum test, $p>0.05$ in all groups.

of the lens care solutions at that time, neutralizing agents such as catalase or platinum were developed eventually. The hydrogen peroxide without preservative became better adapted, although some catalase still contained thimerosal as preservative. Hydrogen peroxide is toxic to the ocular tissue and it must be neutralized before the contact lens can be inserted on eye. The residual hydrogen peroxide after neutralization is our main concern. It has been reported that low concentrations of hydrogen peroxide could induce cataract and endothelial decompensation on animal stusies[6,7]. Retraction and death of the epithelial cell occurred within 12-24 hours after exposure to 1ppm hydrogen peroxide in serum free medium[8]. In our study, three different hydrogen peroxide systems with different neutralization methods revealed no influences on corneal epithelial healing rate. This meant that the residual hydrogen peroxide, if any, was not toxic to the cornea. The neutralizing effect will decay due to the repeat uses of platinum disc and the deposition of contact lenses. Old lenses and used disinfecting systems should be used more cautiously.

The multipurpose solution must be strong enough for disinfection yet mild enough for the corneal safety. The preservative and disinfecting agents play the main role. Hexidin is a chlorhexidine and thimerosal preserved saline storage solution. Chlorhexidine was the first biguanide used in lens care and it was among the first preservatives used for hydrogel lens solutions. In the concentration used for lens care, it exerts no significant effect on yeast and fungi. Combining with thimerosal, it is potent enough to kill or inhibit a broad spectrum of microorganisms. The major problem of this agent is that of the absorptive or binding ability of most hydrogel materials. It is minimally bound to the clean lens. It is implicated as a sensitizing agent, which leads to irritation and hypersensitivity in chronic use. Hexidin in our study showed no influence on corneal epithelial wound healing.

Being used as a disinfectant in Opti-free, polyquad is a large polymeric molecule that resists diffusion into the lens matrix to minimize the toxic or hypersensitive reaction. In our study, it showed a borderline influence to the corneal epithelial wound healing. The Tripathi's study stated that 0.001% Polyquad had no discernible effects on cytokinetic movement of the corneal epithelium[8].

Polyhexanide is the preservative currently used by Bausch and Lomb. Its particular application in this usage is its antimicrobial action even in high dilution. In contact lens solution it is commonly used at a strength of 0.00005%. Tripathi found there was no effect on corneal epithelial cytokinetic movement or the mitotic activity at this concentration[8]. Double the concentration still possessed no cytotoxicity on corneal epithelium[9], though it might be toxic to the rabbit corneal epithelium when 30-50 times greater than the above concentration used for rigid gas permeable lens solution[10]. In our study B&L Sensitive Eyes multipurpose solultion showed a good result for epithelial healing.

Most of the laboratiories paid their attention to the cleaning and disinfecting effects of the lens care solutions. Few studies were concerned about the toxicity. Vaughan and Porter reported an in vitro method for assessing the toxicity for soft contact lens care solutions with the cell culture technique. Their study showed the benzalkonium chloride was particularly toxic to the mammalian cell and should be rinsed off before contact lens insertion. Benzalkonium chloride was seldom used in the multipurpose solution and was not included in any solutions in this study[11]. Bergmanson studied the ocular response to the multipurpose contact lens care solutions, they used eight ocular function measurements as clinical assessment, and found the multipurpose solution was clinically equivalent to physiological saline[12]. In our study, it also revealed that none of the care solutions caused wound healing disturbance effect when compared with the BSS. Our conclusion is that the contact lenses treated in the above solutions should be safe to apply on eye without rinsing. Our study was performed in vitro and on the pig eyes, and it was a short-term study to evaluate the acute toxicity. The long-term effect on human and in vivo may need further study.

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軟式隱形眼鏡保養藥水對於豬角膜表皮傷口癒合之影響

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隱形眼鏡藥水必須具備效率、安全及方便三大要素。藥水中的殺菌劑可殺死微生物，但也會對眼球表面造成傷害。多功能藥水強調其方便性，因此鏡片取出後，不用生理食鹽水沖洗，而直接戴到眼睛上。大量的藥水含在鏡片表面或裏面，直接接觸到眼睛，於軟式隱形眼鏡由於其高含水量，此種情形更加明顯。雙氧水會損傷眼球表面，因此需先中和後，鏡片才能配戴，中和不完全殘留之雙氧水，也可能傷害到眼球表面。我們以豬眼睛爲材料，在體外測試這兩類藥水對於角膜表皮傷口癒合之影響。

雙氧水類的藥水包括AOSEPT、Oxysept和Contopharma-Peroxide-System。先依照指示的方法中和後，再滴於角膜傷口上。多功能藥水包括Opti-free、Hexidin和Bausch & Lomb則直接滴於角膜傷口上。豬眼球由屠宰場收集後，置於冰箱中帶回，先清除結締組織及結膜後，浸入PVP-iodine和Gentamycin中消毒。我們以準分子雷射於角膜表皮正中央切削一個直徑1.5mm，深度70μm之傷口，然後以一灌注系統灌注TC-199培養液，維持眼球於培養箱中，保持角膜朝正上方。於裝置完成後，立刻以TC-199培養液潤濕角膜表面，然後分別於1、3、5小時後，點實驗藥水或BSS共三次於傷口上。於24小時後傷口以螢光試劑染色，以藍色燈光檢查其傷口之大小，依其癒合程度給予0至3分，0分爲完全癒合。所得之分數以Mann-Whitney rank-sum test檢算。統計上所有隱形眼鏡藥水和BSS比較，均無延遲傷口癒合之現象，顯示這些隱形眼鏡藥水對於豬眼球角膜表皮於短時間內並不具毒性。

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