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Determination of Deproteinization Effect of 3N Contact Lenses Cleaner

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Abstract: 3N Contact Lenses Cleaner is a device utilized in effectively removing the protein deposition on contact lenses. It is independently developed by 3N Biological Technology Co., Ltd (3N Tech). Using the principle that proteins move in the electric field, 3N Contact Lenses Cleaner is able to adsorb proteins from the surface as well as the tiny holes of contact lenses, therefore, removes the protein deposition from the contact lenses effectively. In this study, we immersed contact lenses in artificial tears, and then proved 3N Contact Lenses Cleaner's deproteinization effect with a qualitative detection using Coomassie Brilliant Blue G-250's feature of indicating proteins. Meanwhile, we determined the device's protein elution effect with Bradford method. The result indicates that 3N Contact Lenses Cleaner can distinctively remove the protein deposition from contact lenses.

Keywords: 3N Contact Lenses Cleaner, Protein Movement, Deproteinization, Bradford Method

1. Introduction

Unlike traditional frame glasses, the full use of hydrophilic contact lenses includes care for lenses. This step is necessary because hydrophilic contact lenses are composed of Hema materials with high water content, lenses in the process of wearing, continuous contact with tears, the protein in tears inevitably deposited into the lens surface and interior, reduce the oxygen permeability of the lens [1]. In order to reduce the impact of this disadvantage, it is necessary to remove the deposited protein, the current main method is the use of care fluid and the addition of special protein-removing enzyme tablets, and they mainly clean and disinfect the lens [2]. Furthermore, Mechanical rubbing is difficult to completely remove the deposition of proteins deposited inside the lens, but it can still exert some effect on the removal of the surface sediments (including proteins and other precipitates). However, it has caused great damage to the lens [3]. Previous studies have evaluated the effect of the traditional nursing solution on protein removal [4-8]. However, the residue of contact lenses remains an important problem for the wearer, and further development of new methods for removing protein residues is needed.

Contact Lens, medically also addressed as 'Corneal Contact Lens', is quite popular, especially among young people, for its beauty and convenience. However, the proteins secreted from human tears and other impurities deposited on both sides of the surface of the lens as well as in its stroma are considered significant causes of a series of complications [9]. 3N Contact Lenses Cleaner is independently developed by Suzhou 3N Biological Technology Co., Ltd (3N Tech). It is made of medical-grade ABS plastic, which makes it safe to use, heat and cold resistant, and impact resistant. It utilizes the principle of which proteins move in the electric field, to adsorb the proteins and other impurities deposition from both the surface and the tiny holes of the lenses, thus greatly reliefs the discomforts of the wearers, such as eye dryness and redness, reducing the chances of infection.

The study firstly detected 3N Contact Lenses Cleaner's adsorption of proteins qualitatively, and set up an experimental method of examining the movement of proteins in the electric field of 3N Contact Lenses Cleaner. Secondly, with Bradford method's quantitative determination of proteins, we carried out deproteinization tests with both 3N Contact Lenses Cleaner and contact lenses solution commonly sold on

the market, and then examined the effect of 3N Contact Lenses Cleaner's clearance on proteins attached to contact lenses with Bradford method [10]. Through this study, we further proved 3N Contact Lenses Cleaner's deproteinization effect on contact lenses.

2. Materials and Methods

2.1. Materials

(1) Preparation of artificial tears: lysozyme (Sigma), BAS, $Na_2HPO_4.2H_2O$, $CaCl_2.2H_2O$ and NaCl compounded according to conventional method, adjust the pH to 7.0.

(2) Contact lenses solution and contact lenses are both commonly available in the market.

(3) Apparatus: 3N Contact Lenses Cleaner, small petri dish, tweezers, slides, 1.5ml centrifugal tube, incubator, Microplate Reader etc.

(4) Reagents: Quick start Bradford Protein Assay Kit (Bio-Rad 5000201), 6 X SDS Loading buffer (Thermo Fisher R0891).

2.2. Methods

2.2.1. Qualitative Experiment on Deproteinization

(1) Prepare 0.1mL of Iysozyme solution with the concentration of 0.5 mg/mL, add in 0.02 mL of 6 X SDS Loading buffer and then fully mix, boil the mixture for 5 minutes at the temperature of 95°C, and then mark the protein with dye.

(2) Add 2 mL of contact lenses solution in the grooves of 3N Contact Lenses Cleaner, take 0.05 mL of the dyed protein from (1) and put it in the groove with the negative electrode, turn on electric power, and observe the movement of the dyed protein in the electric field of 3N Contact Lenses Cleaner.

2.2.2. Quantitative Experiment of Deproteinization

(1) Control group: contact lens solution mark as CK1

Treated group: immerse contact lenses in prepared artificial tears as well as in contact lenses solution for 24h, 48h, and 72h, take them out and wash three times with double distilled water.

Clean the contact lenses with 3N Contact Lenses Cleaner for 30 seconds, then mark them as M-24h, M-48h and M-72h; immerse the contact lenses in contact lenses solution overnight, then mark them as N-24h, N-48h and N-72h.

(2) Determination of Protein:

Conduct quantitative determination on the protein aqueous solution from (1) with Bradford reagent (Bio-Rad).

3. Results

3.1. Protein Moves in 3N Contact Lenses Cleaner's Electric Field

The principle 3N Contact Lenses Cleaner utilizes is that proteins are of colloidal properties and carry negative charge in certain environment, which enables them to move towards the positive pole in an electric field. In his study, lysozyme, which is the main composition of dacryolin, is taken as research subject, to simulate the movement of dacryolin in 3N Contact Lenses Cleaner. The bromophenol blue in 6 X SDS Loading buffer can indicate the movement of protein, and glycerol adds weight to the protein, which on one hand slows down its movement in contact lenses solution, on the other hand simulates proteins' resistance on contact lenses. When power was on, it could be observed that the proteins moved to the positive pole in 3N Contact Lenses Cleaner (Figure 1). Picture 1A shows the 05 mL of proteins dyed with bromophenol blue in 3N Contact Lenses Cleaner's groove with the negative pole slightly movedtowards the positive pole when electric power was on. Shortly after, numerous proteins moved to the positive pole from the negative side (Figure 1B). With the passage of time, the color of the bromophenol blue on the negative side became lighter and lighter, but the color on the positive side did not get dark. However, the movement of proteins from the negative side to the positive side could still be observed between the grooves (Figure 1C). Not a large quantity of bromophenol blue dyed proteins was observed in the positive groove, for one thing, the color of bromophenol blue had been diluted by the large amount of contact lenses solution in the process, for another, it's quite possible that the proteins did not stay in the groove, but were adsorbed to the positive pole, which needs further experiment to confirm.

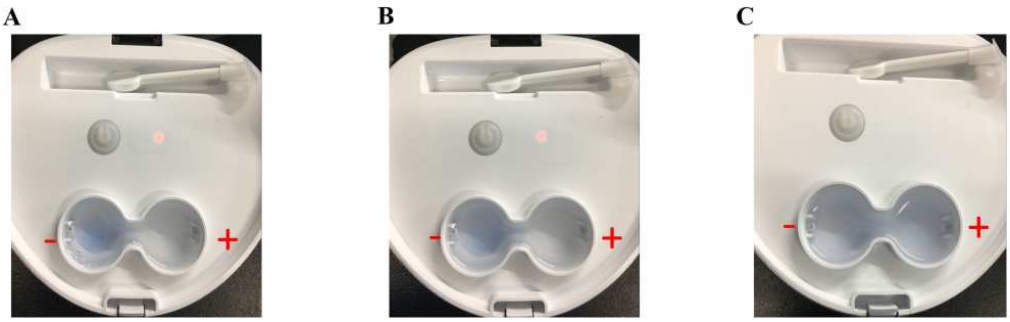

Figure 1. *Movement of proteins in the electric field.*

3.2. 3N Contact Lenses Cleaner's Adsorption of Proteins

This study shows that proteins move in the electric field of 3N Contact Lenses Cleaner. To further illustrate 3N Contact Lenses Cleaner's adsorption of proteins, we analyzed the whereabouts of the moving proteins (Figure 2). When the

above experiment finished, we emptied the contact lenses solution from 3N Contact Lenses Cleaner and washed 3N Contact Lenses Cleaner with distilled water. White remains of colloidal substance were found on the positive pole, and no remains were observed by naked eye on the negative pole (Figure 2A). To further analyze the composition of the remains, we added 5 ul of Bradford 1x Dye Reagent to both the positive and negative poles. Bradford 1x Dye Reagent contains Coomassie brilliant blue G-250 which turns blue-green when encounters protein (Compton and Jones 985). The result showed that the color of the positive pole turned blue, which proved the existence of proteins, but there were no changes on the negative pole (Figure 2B). In addition, before adding Bradford 1x Dye Reagent to the electric poles, we took some of the colloidal substance from the positive pole and placed it on the slide, added Bradford 1x Dye Reagent, and the colloidal substance turned blue again (Figure 2C), indicating there were proteins in the colloidal substance.

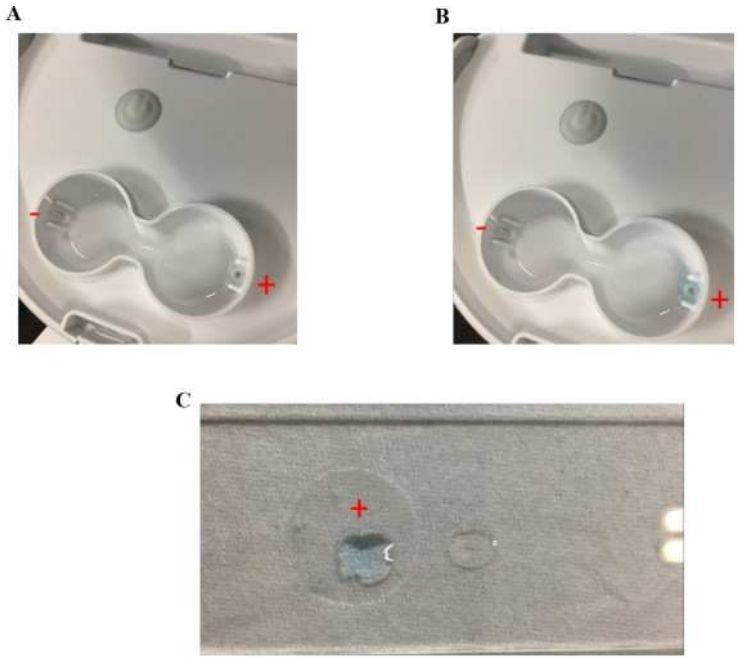

Figure 2. *Positive Pole Adsorbs Moving Proteins.*

The above experiment indicated that proteins move in the electric field of 3N Contact Lenses Cleaner from the negative pole to the positive pole, and were adsorbed to the positive pole.

3.3. Determination of 3N Contact Lenses Cleaner's Efficiency of Deproteinization

To study 3N Contact Lenses Cleaner's efficiency in removing the protein deposit from the contact lenses, we immersed contact lenses with artificial tears and then detected the effect of deproteinization with Bradford quantitative method.

3.3.1. Preparation of Standard Curve

We perform quantitative test of protein on artificial tears with Bradford method, and the standard curve is shown in Figure 3.

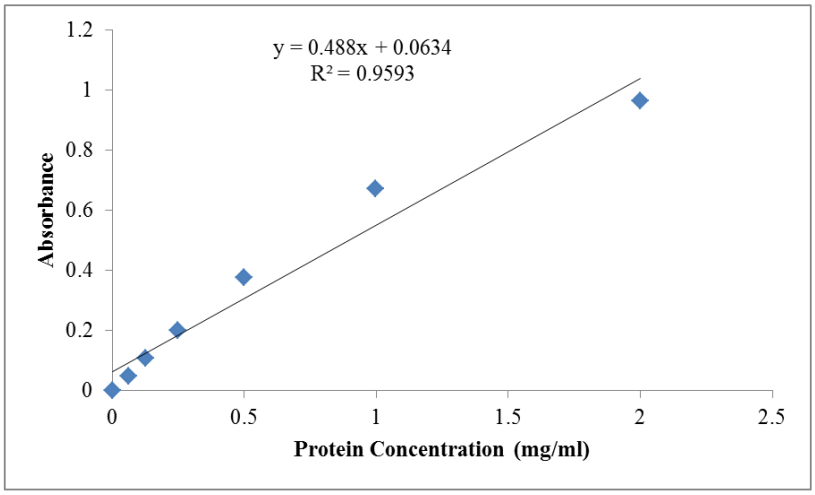

Figure 3. *Standard Curve.*

3.3.2. Determination Result

We carried out quantitative test of protein separately on the liquid from 3N Contact Lenses Cleaner in which contact lenses were cleaned and the liquid of contact lenses solution in which contact lenses were immersed. The results are as the following table 1. From table 1, it can be read that the corresponding Protein Concentration of each treated group is as table 2.

Table 1. *Absorbance of protein.*

	CK1	M-72h	M-48h	M-24h	N-72	N-48	N-24
Absorbance	0.3716	0.4812	0.4787	0.4643	0.3784	0.3728	0.3822
Protein Concentration	0.632	0.856	0.851	0.822	0.646	0.634	0.633

Table 2. *The quantification of protein eluted by 3N.*

Treatment	M-72h	M-48h	M-24h	N-72	N-48	N-24
Elution	0.224	0.219	0.19	0.014	0.002	0.001

The 'eluted protein concentration' in Table 2 refers to the proteins eluted by 3N Contact Lenses Cleaner or contact lenses solution, which equals the protein concentration from chart 1 minus the concentration of CK1. Due to the fact that contact lenses solution itself contains proteins, as there was absorbance when the solution was measured alone, to calculate the amount of proteins eluted, we need to subtract the background value of the solution.

4. Conclusions

We used bromophenol blue's feature of indicating proteins, firstly observed proteins' movement in 3N Contact Lenses Cleaner's electric field, secondly, with Coomassie brilliant blue G-250's nature of dyeing proteins, we detected the gathering of proteins at the positive pole, and confirmed 3N Contact Lenses Cleaner truly is effective in removing proteins. Meanwhile, with Bradford quantitative test, we analyzed 3N Contact Lenses Cleaner's efficiency in deproteinization. The eluted protein concentration of the liquid used for deproteinizing contact lenses in 3N Contact Lenses Cleaner is much higher than that of the liquid that was not used in the device. This means that the protein deposit on contact lenses have been removed effectively. However, due to our current lack of apparatus for examining the remains on contact lenses, the result of the experiment is not enough to prove whether other remains of impurity are also completely removed, which requires follow-up experiments to prove.

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