Quantitative Evaluation of Human Lens Epithelial Cell Viability and Cytolysis by Distilled Water Ex Vivo

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Research Article

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Abstract

Purpose: To investigate the role of distilled water (DW) in isolated human lens epithelial cells (LECs) viability and lysis ex vivo.

Methods: After immersion in DW or balanced salt solution (BSS) for 1-, 2-, and 3-minutes, respectively, the cell viability of LECs was quantitatively evaluated. In addition, the capsule samples soaked in DW or BSS for 1-, 2-, and 3-minutes were combined with rinse for 1 minute to analyze the difference of LECs shedding percentage in each subgroup. The histopathological changes of the samples after treating were observed.

Results: The percentage of LECs shed in DW immersion combined with rinse was significantly higher than in DW immersion alone (*p* all <0.001). In the subgroup soaked in DW for 3 minutes, the death number, mortality, and the percentage of cell shedding of LECs was the most (*p* all <0.001). The histopathological changes showed that the cell destruction in the DW subgroup for 1-, 2-, and 3-minutes, and the transmission electron microscope results showed that the cells were partially detached from the capsule in the DW 3 minutes subgroup.

Conclusions: Soaking in the DW can cause LECs death, and DW immersion combined with rinse was an effective method to remove LECs. The histopathology changes of treated DW suggested cellular necrosis was one type of LECs death mechanism.

Introduction

Lens capsule opacification, as a common complication after cataract surgery, will cause decreased postoperative vision and affect the effect of surgery. At present, it is believed that lens capsule opacification is the stimulation of surgical trauma, which leads to the proliferation, migration, and epithelial-mesenchymal transition (EMT) of the residual lens epithelial cells (LECs) in the periphery and equatorial part of the lens[1]. Lens capsule opacification includes anterior capsule opacification (ACO), leading to anterior capsular contraction and IOL deviation[2], and posterior capsular opacification (PCO) blocking the visual axis, resulting in vision decreased[3]. The rate of anterior capsule fibrosis and phimosis after cataract surgery was about 0.47-3.3%[4]. During the follow-up of 3-5 years after cataract surgery in adults, the incidence of PCO was about 20-30%[5]. The LECs of children display higher proliferation and migration properties than those in adults[6], and almost 100% children develop lens capsule opacification after surgery, which has a severe impact on visual development[7].

Lens capsule opacification is usually treated with neodymium: YAG (Nd:YAG) laser capsulotomy or secondary surgical capsulotomy. The former is relatively simple and convenient, but there is a risk of complications[5, 8]. The latter is an invasive method. Hence, there is a strong need to prevent lens capsule opacification after cataract surgery. Currently, there are two possible preventive directions against lens capsule opacification: inhibiting the proliferation, migration, and EMT of LECs, and clearing the LECs as

far as possible. The former includes using drugs to inhibit the proliferation, migration, or EMT of LECs[9], implantation of hydrophobic sharp-edged IOLs[10] or a thick endocapsular open ring to inhibit the migration of LECs to the posterior capsule[11]. However, LECs remain within the capsular bag and the causative reason can't be eliminated[12]. The latter approach is mainly via the combination of drugs and surgical techniques to clear the residual LECs in the capsule as far as possible[13, 14], which is an active area of research. However, the potential toxic effects of many drugs on intraocular tissues limit their clinical application[15, 16].

Distilled water (DW) induces cell lysis by hypoosmotic pressure[17], which has no chemical toxicity to intraocular tissues and can be neutralized quickly by balanced salt solution (BSS). However, the results of current human clinical trials of DW are divergent. The effectiveness and optimal duration of action of DW on LECs are not completely clear. For this purpose, we carried out this study, taking the isolated anterior capsule of patients with age-related cataract (ARC), to explore the role of DW in LECs viability and the clearance effect of DW combined with or without BSS on LECs.

Materials And Methods

Patients

Samples were obtained after approval from Ethics Committee of Nanjing Drum Tower Hospital and in accordance with the guidelines of the Declaration of Helsinki. All patients signed an informed consent prior to surgery. The anterior capsules (5.0mm-5.5mm in diameter) were obtained from uneventful cataract surgery (age: 69.40±11.52, n=156, ARC) in Department of Ophthalmology of Nanjing Drum Tower Hospital.

Methods

The collected anterior capsules were quickly split into two pieces (312 small pieces of anterior capsule samples). 282 pieces of these samples were divided into 6 groups: negative control group (23 small pieces, no drug treatment), positive control group (10 small pieces, fixed directly with 4% tissue cell stationary fluid), BSS group, DW group, BSS with rinse group, and DW with rinse group. The samples soaked in BSS or DW alone were used to investigate the effect of DW on LECs viability, and the capsule samples soaked in DW or BSS combined with rinse were used to explore the clearance effect of DW on LECs. 30 pieces were used for histopathological examination.

There were 124 small pieces anterior capsule samples soaked in BSS or DW for 1, 2, or 3 minutes, respectively (61 pieces in BSS, 63 pieces in DW). Samples in the two control groups and BSS/DW group were stained with trypan blue-eosin to measure cell viability. Photographs at 40× under the light microscope were used for the next procedure. 10 nonoverlapping images were selected from each group at nuclear level and cell contour level, respectively, by a light microscope at 400×. The LECs density (per

mm^2), the number of LECs deaths (per mm^2), and LECs mortality (%) were calculated by Image J (Fig. 1). The calculation formulas were as follows:

$$\text{the LECs density (per } mm^2\text{)} = \frac{\text{sum of cells per square in 10 images}}{0.005\times5\times10}$$

$$\text{the number of LECs deaths (per } mm^2\text{)} = \frac{\text{sum of blue nuclei in 10 images}}{\text{image area at 400}\times\times10}$$

$$\text{the LECs mortality (\%)} = \frac{\text{thenumberofLECsdeaths}}{\text{theLECsdensity}}$$

63 pieces were immersed in BSS for 1, 2, or 3 minutes, respectively, and then the group was rinsed with BSS at the height of 70cm bottle for 1 minute. 62 pieces were in DW immersion for 1, 2, or 3 minutes, respectively, and then the group was rinsed the same as mentioned above. After treatment, 125 pieces mentioned above were stained with trypan blue-eosin, and were photographed under a light microscope at 40×, to compare with the negative control, BSS or DW group. About 5 to 6 pictures can be stitched into an intact piece of capsule. Then the shedding percentage of LECs (%) was calculated by Image J. Detailed calculation method was provided in Figure 2 and the shedding percentage of LECs (%) was calculated by the formula

$$=1-\frac{\text{LECsareaaftertreated}}{\text{total area of spiced capsule}}$$

The staining step of trypan blue-eosin was: 1) 0.04% trypan blue solution for 1 minute, 2) gentle irrigation of BSS for twice, 3) tissue cell fixation solution for 10 minutes (except the positive control group), 4) rinsing for 3 minutes in phosphate buffered saline (PBS), 5) staining with eosin for 30 sec, 6) gentle washing with tap water and left to dry at room temperature.

Histopathological examination was performed on 30 pieces samples, which were divided equally into 5 groups (the negative control, BSS 3 min group, DW 1 min group, DW 2 min group, and DW 3 min group). After treatment, 3 pieces from each group (n=15) were stained with hematoxylin and eosin (HE) and then examined by light microscope. The others were examined by transmission electron microscopy (TEM).

For light microscopic observation,the samples after treatment were immersed in fixative solution for over 24 hours, dehydrated, dipped in wax, embedded, sliced, regular HE stained, and sealed with neutral gum in turn, and then were observed under light microscope.

For TEM observation, treated samples were fixed with 2.5% glutaraldehyde, stored at 4℃ for more than 12 hours postfixed with 1% osmiumtetroxide, washed three times with PBS, dehydrated with a graded ethanol series, treated with pure acetone, treated with a mixture of embedding agent and acetone (v/v=3/1) for 3 hours, embedded, sectioned with an ultrathin slicer, and double stained with lead citrate and uranyl acetate in turn. And then, the sections were observed and photographed after drying.

Statistical analysis

Statistical comparison was estimated by a one-way analysis of variance (*ANOVA*) followed by a *Dunnett-t* test for comparing all groups with the control group. *Tukeys* test or *Games-Howell* test was performed for pairwise comparison. Comparisons between groups or subgroups were performed by paired *t*-test. Statistical tests were two-side with a significant level of 0.05.

Results

LECs viability

The anterior capsule LECs of the normal ARC patients in the negative control group exhibited a regular, polygonal-like shape with a small number of dead cells. In the positive control group, blue cell nuclei were distributed everywhere within the field of view and all cells were dead. The LECs in BSS group with distinct cellular borders showed no significant differences compared with the negative control group. In the 3 subgroups in DW group, the number of LECs expansion was becoming greater as the duration time increased, and the dead cells had indistinct cell boundaries (Fig. 3).

The differences in the LECs density, the LECs death number, and the LECs mortality among the negative control group, BSS, or DW group were significant (Welch F=12.133, 75.887, 85.255, p <0.001). The decrease in the LECs density in DW immersion for 2- and 3-mins represented statistically significant differences compared with the negative control group (p <0.05, p <0.001). The increase of the LECs death number and the LECs mortality in DW immersion for 1-, 2-, and 3-mins showed statistically significant differences compared with the negative control group (p all <0.001), indicating that DW had the capacity to destroy LECs (Table 1).

Table 1
Lens epithelial cells (LECs) viability of treated groups

Groups	Pieces of anterior capsules (piece)	LECs density (per mm^2)	Number of LECs deaths (per mm^2),	LECs mortality (%)
Negative control group	23	3829.08±519.16	263.01±142.85	7.16±4.28
BSS for 1 min	20	4054.63±489.64	280.99±124.24	7.03±3.14
BSS for 2 mins	21	3985.13±687.90	292.07±129.35	7.30±2.76
BSS for 3 mins	20	4114.35±503.56	318.49±176.66	7.72±4.02
DW for 1 min	20	3501.20±281.99	1045.47±496.70[b]	29.82±13.83[b]
DW for 2 mins	23	3329.59±370.95[a]	1667.40±517.76[b]	50.51±15.83[b]
DW for 3 mins	20	2898.03±845.04[b]	1956.27±434.45[b]	70.05±15.40[b]

The superscript represented that the difference between the group and the negative group was significant by *Dunnett-t* test, letter “a” meant the p values were less than 0.05, and letter “b” meant the p values were less than 0.001.

When the immersion time was the same, the LECs death number and the LECs mortality in DW group were greater than those in BSS group (p all <0.001). In DW group, the LECs death number and the LECs mortality increased with longer immersion times. These data suggested that DW had a stronger destructive ability on LECs than BSS and the ability increased over time, that was, DW was more effective than BSS for disruption of LECs (Fig. 4).

Percentage of LECs shedding

Only a small amount of LECs shedding was detected in the negative control group, BSS group, and BSS combined with rinse group. A fraction of LECs shedding was observed in DW group. The LECs shedding was evident in DW combined with rinse group, and the range of LECs shedding was larger as DW immersion time increased (Fig. 5).

The differences showed no significant differences in BSS group, BSS combined with rinse group, and the negative control group (p >0.05). There were significant differences in the percentage of LECs shedding among DW group, DW combined with rinse group, and the negative control group (Welch F=386.267, p <0.001). And the percentage of shedding LECs in DW group, DW combined with rinse group was higher than that in the negative control group (p all <0.001). These data gave indications that DW could cause cell death and contribute to LECs clearance (Table 2).

Table 2
Percentage of LECs shedding in treated groups

Groups	Pieces of anterior capsules (piece)	Percentage of LECs shedding (100%)
Negative control group	23	11.04±4.85
BSS for 1 min	20	10.88±5.65
BSS for 2 mins	21	11.43±4.95
BSS for 3 mins	20	18.80±7.97
DW for 1 min	20	36.40±15.83[a]
DW for 2 mins	23	41.29±17.55[a]
DW for 3 mins	20	46.22±18.88[a]
BSS for 1 min combined with rinse	20	17.77±10.25
BSS for 2 mins combined with rinse	22	19.81±7.37
BSS for 3 mins combined with rinse	21	19.07±6.70
DW for 1 min combined with rinse	20	60.02±14.80[a]
DW for 2 mins combined with rinse	22	81.31±13.01[a]
DW for 3 mins combined with rinse	20	94.12±4.86[a]
The superscript “a” represented that the difference between the group and the negative group was significant by *Dunnett-t* test, and the p values were less than 0.001.		

When the time of soaking was the same, the average percentage of shedding LECs in DW combined with rinse group was more than that in DW group (p all <0.001). In subgroups of DW combined with rinse group, the percentage of shedding LECs increased with immersion time (comparisons among subgroups: Welch F=51.990, p <0.001, pairwise comparison between subgroups: p all < 0.005) (Fig. 6).

Microstructure of treated LECs

In the negative control group and BSS immersion for 3 mins group, the LECs with regular cell shape and round nuclei were arranged in a single layer and adhered to the capsule. In DW immersion for 1-, 2-, and 3-mins subgroups, the cell integrity disruption of LECs, the cytoplasmic outflow of LECs, and some LECs shedding were observed in DW immersion for 2- and 3-mins subgroups (Fig. 7).

Ultrastructure of treated LECs

In the negative control group and BSS immersion for 3-mins group, the LECs appeared intact cellular morphology, round or round-like nuclei, complete nuclear membranes, clear nucleolus, evenly distributed chromatin, and normal morphology of cell organelles. And the interconnections of LECs showed finger-like protrusions and LECs arranged in a single layer and adhered to the capsule. In DW immersion for 1-, and 2-mins groups, it could be seen cytolysis, nuclei with regular morphology, cytoplasmic destruction, swollen cellular organelles, and outflow of the cytoplasm. Disruption of intercellular junctions and loose cell-to-capsule junctions were also observed. In DW immersion 3-mins group, we could see the efflux of intercellular substances, decreased cell volume, nuclear deformation, and multiple sharp neurites, cytoplasmic destruction, and swelling of organelles. Disrupted intercellular junctions and partial separation of LECs to capsule were also viewed (Fig. 8).

Discussion

DW induced cell rupture in hypoosmotic conditions, which could theoretically reduce the survival ratio of LECs. A previous study showed that the LECs exposure to DW for 1 min were markedly swollen with grossly morphologically intact cell membranes, and approximately 2 mins of exposure to DW, the complete cell lysis could be obtained[17]. Another study suggested that exposure to DW for 1 min, the LECs kept intact, cell membranes markedly swollen, and by 2 mins, no intact cells were visible[18]. Regrettably, no quantitative analysis was performed in the 2 studies of Crowston et al., the results were observed under microscope.

In this study, quantitative analyses and accurate calculations of the LECs density, the LECs death number, and the LECs mortality were conducted to obtain more precise results by trypan blue-eosin staining and photographing under a microscope[19], which could provide a more comprehensive assessment of the effects of DW on LECs viability and clearance. The density of cell population in cataractous lenses varied greatly. The mean number was around 4000/mm^2, but there were lenses with even lower cell counts[20]. In the negative control group of our study, the LECs density was 3829.08±519.16 mm^2, which was similar to the conclusion of the study of Laspias et al.[20]. Therefore, our calculation method could be considered reliable.

Quantitative analysis showed that the LECs mortality was 70.05% in DW immersion for 3 mins, which was 2.35 times higher than that in DW immersion for 1 min. This result was similar to that of Rękas et al.[21]. There was 70.8% cytolytic destruction of LECs after 3-minute exposure to DW, which was 3.5 times higher than 1-minute exposure to DW[21]. We also set a negative control group, as well as BSS group acting for the same treatment time, to compare with the DW group. The result suggested that soaking in DW for 2 or 3 mins could effectively cause LECs death, and the effect of DW immersion for 3 mins was better. Naturally, there were different study findings. In the study of Duncan et al., there was 50% cell survival after FHL124 cells exposed for 2 minutes to DW[22]. This could be due to the different materials and different counting methods. In the study of Rabsilber et al., it suggested that using DW was unable to

reduce PCO development significantly[23], which was different from ours. This might be caused by the insufficient acting time of DW.

During cataract surgery, the whole capsular bag should be rinsed with irrigation/aspiration (I/A) to remove the lens cortex and viscoelastic agent. Therefore, it might be considered to introduce DW into the capsule combined with stimulated I/A, which could not only fully exploit the capacity of DW to destroy cells, but also the neutrality against DW and clear the LECs with loosened junctions to capsule. In this study, BSS at the height of 70cm was used to rinse the capsule for 1 minute to simulate I/A during the cataract surgery. Results indicated that DW immersion combined with rinse contributed to LECs clearance, and the most effective exposure time was 3 mins. Some research suggested that 360-degree anterior capsular polishing alone was unable to reduce the incidence of PCO[24], meaning that mechanical anterior capsular polishing alone was unable to clear all LECs in the capsular bag. Our results indicated that rinsing combined with exposure to hypotonic DW could effectively clear LECs.

To minimize the side effects on intraocular tissues, additional protective measures might be necessary. The sealed capsule irrigation (SCI) was designed to temporarily seal the capsular bag to allow drug injection and saline flush to protect the intraocular tissues[8]. Rękas et al. infused DW in SCI for 3 mins after the removal of cortical material and rinsed the intracapsular space with 0.9% NaCl[25]. They recognized that DW irrigated for 3 mins reduced PCO in the long-term follow-up, which was more effective than anterior capsule mechanical cleaning[25]. After nucleus removal and cortical aspiration, Zhang et al. filled the anterior chamber with a continuous infusion of sterile air, and then injected DW or BSS into the capsule for 3 mins and followed by irrigating the capsular bag with BSS with the assistance of vitrectomy machines[13]. It was found that the technique could significantly prevent capsular fibrosis and PCO[13]. However, SCI was inapplicable to patients with deep anterior chamber or a small pupil[23], and unsuitable to microincisional (1.8 mm or 2.2 mm) cataract surgery[13]. What's more, additional surgical techniques and equipment were necessary in the forementioned methods, which limited their popularized. DW combined with rinse could effectively remove LECs to prevent the occurrence of capsule opacification after cataract surgery, but how this technique could be applied in the clinic still needed further exploration.

Cell death exists in many different forms, such as necrosis, apoptosis, necroptosis, pyroptosis, and autophagy. Necrosis, a common form of pathological cell death, can be identified via hitstopathologic changes in both nuclei and cytoplasm. Our results suggested that soaking in DW not only induced LECs death, but also contributed to LECs clearance. We further considered how the LECs might change as soaking in DW, and cytological observations were carried out on treated LECs. HE staining showed cytolytic destruction and partial shedding of LECs in DW immersion for 2- and 3-mins subgroups. In the study of Rękas et al., basement membrane and LECs were occasionally seen in the specimens prepared for microscopic examination[21]. The results were the same as our light microscopic results. TEM results showed LECs destruction in DW immersion group, loose junctions between cells and capsule in DW immersion for 1- and 2-mins subgroups, and partial separation of LECs from capsule in DW immersion

for 3-mins subgroup. The lysis and rupture of the cell membrane, another symbol of necrosis, could be found in our HE staining and TEM results. Therefore, it can be concluded that DW can cause LECs death via necrosis. Our results suggested that DW could not only cause LECs death, but also loosen the junctions of cells with the capsule, which could shed LECs from capsule partially. Therefore, the shedding percentage of cells increased after soaking in DW. All the above histocytological findings were in agreement with the differences of LECs shedding percentage shown by trypan blue-eosin staining.

Nevertheless, the present study still had some limitations. First, DW could cause LECs death but not enough to kill all LECs. Considering DW frequently was used as a solvent, we did not know whether the trial for combining with other drugs to destroy LECs more fully could have been attempted[24]. Second, although DW combined rinse was effective on cell shedding but couldn't make all cell shedding. To this point, part of the reason lied in the fact that partial folding led to some LECs failing to be cleared when the capsule was rinsed with BSS. Third, LECs of anterior capsules were studied in this study, while the cellular morphology and function of LECs of the anterior and equatorial lens capsules were not the same[26]. Therefore, further animal experiments were necessary to explore the clearance effect of DW on LECs of the equatorial lens capsule. The histopathological changes of treated LECs with DW indicated cellular necrosis, which suggested that there were other operative mechanisms of DW aside from the hypoosmotic mechanism. All these aspects await further investigation.

It was concluded that DW immersion can cause LECs death and DW immersion combined with rinse was an effective method to remove LECs. The histopathology changes of treated DW suggested cellular necrosis was one type of cell death. All of these provided theoretical grounding for preventing lens capsule opacification after cataract surgery via DW.

Declarations

Availability of data and materials

The data are available from the corresponding author upon reasonable request.

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Contributions

Wen-Wen Zhang, Rong-Pei Zhang and Ya-Jun Liu contributed equally to this work. Zheng-Gao Xie, Wen-Wen Zhang, Rong-Pei Zhang and Ya-Jun Liu designed the study; Rong-Pei Zhang, Ya-Jun Liu, Fei-Fei Chen, Zi-Fang He and Si Zhang conducted the study, collected data; Wen-Wen Zhang, Rong-Pei Zhang and Ya-Jun Liu analyzed and interpreted data; Wen-Wen Zhang and Rong-Pei Zhang preparation and writing the manuscript; Zheng-Gao Xie and Wen-Wen Zhang review and approval of the manuscript.

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Ethics declarations

Ethics approval and consent to participate

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Nanjing Drum Tower Hospital, China. Written informed consent was obtained from each patient prior to participation in the study.

Consent for publication

We have obtained explicit written informed consent to publish all data (including individual details, images or videos) related to the study from patients or their parents.

Competing interests

The authors declare that they have no competing interests.

References

1. Fișuș AD, Findl O. Capsular fibrosis: a review of prevention methods and management. Eye (Lond) 2020; 34(2): 256–262.
2. Pérez-Vives C. Biomaterial Influence on Intraocular Lens Performance: An Overview. J Ophthalmol 2018; 2687385.
3. Chen HC, Lee CY, Sun CC, et al. Risk factors for the occurrence of visual-threatening posterior capsule opacification. J Transl Med 2019; 17(1):209.
4. Liu YC, Wilkins M, Kim T, et al. Cataracts. Lancet 2017; 390(10094): 600–612.
5. Awasthi N, Guo S, Wagner BJ. Posterior capsular opacification: a problem reduced but not yet eradicated. Arch Ophthalmol 2009; 127(4): 555–562.
6. Zhang J, Hussain A, Yue S, et al. Osmotically induced removal of lens epithelial cells to prevent PCO after pediatric cataract surgery: Pilot study to assess feasibility. J Cataract Refract Surg 2019; 45(10): 1480–1489.
7. Trivedi RH, Wilson ME. Posterior capsule opacification in pediatric eyes with and without traumatic cataract. J Cataract Refract Surg 2015; 41(7): 1461–1464.
8. Aslam TM, Devlin H, Dhillon B. Use of Nd:YAG laser capsulotomy. Surv Ophthalmol 2003; 48(6): 594–612.
9. Smith AJO, Eldred JA, Wormstone IM. Resveratrol Inhibits Wound Healing and Lens Fibrosis: A Putative Candidate for Posterior Capsule Opacification Prevention. Invest Ophthalmol Vis Sci 2019; 60(12): 3863–3877.
10. Schartmüller D, Schriefl S, Schwarzenbacher L, et al. Posterior capsule opacification and Nd:YAG laser rates with two hydrophobic acrylic single-piece IOLs. Eye (Lond) 2020; 34(5): 857–863.
11. Pallikaris IG, Stojanovic NR, Ginis HS. A new endocapsular open ring for prevention of anterior and posterior capsule opacification. Clin Ophthalmol 2016; 10: 2205–2212.
12. Balendiran V, Werner L, Ellis N, et al. Uveal and capsular biocompatibility of a new hydrophobic acrylic microincision intraocular lens. J Cataract Refract Surg 2020; 46(3): 459–464.
13. Zhang Q, Huang L, Jin H, et al. Air/fluid-dropping technique for intracapsular distilled water application: a vitrectomy approach for selective targeting of lens epithelial cells. Retina 2019; 39(2): 364–370.
14. Nishi O, Nishi K, Hikida M. Removal of lens epithelial cells following loosening of the junctional complex. J Cataract Refract Surg 1993; 19(1): 56–61.
15. Wertheimer C, Kueres A, Siedlecki J, et al. The intraocular lens as a drug delivery device for an epidermal growth factor-Receptor inhibitor for prophylaxis of posterior capsule opacification. Acta Ophthalmol 2018; 96(7): e874-e882.
16. Sobol EK, Ahmad S, Ibrahim K, et al. Rapidly progressive streptococcus dysgalactiae corneal ulceration associated with erlotinib use in stage IV lung cancer. Am J Ophthalmol Case Rep 2020; 18:100630.

17. Crowston JG, Maloof A, Healey PR, et al. Water modulation of lens epithelial cells during cataract surgery. J Cataract Refract Surg 2003; 29(12): 2464–2465.
18. Crowston JG, Healey PR, Hopley C, et al. Water-mediated lysis of lens epithelial cells attached to lens capsule. J Cataract Refract Surg 2004; 30(5): 1102–1106.
19. Vasavada AR, Cherian M, Yadav S, et al. Lens epithelial cell density and histomorphological study in cataractous lenses. J Cataract Refract Surg 1991; 17(6): 798–804.
20. Laspias GA, Thomopoulou GH, Lazaris AC, et al. Cytomorphometric study of epithelial cells in normal and cataractous human lenses in relation with hyperglycemia. Int Ophthalmol 2016; 36(2): 147–158.
21. Rękas M, Kluś A, Stankiewicz A, et al. Lens epithelial cell damage after exposure to deionized water to prevent posterior capsule opacification after phacoemulsification: an in vitro study. Med Sci Monit 2010; 16(3): BR102-106.
22. Duncan G, Wang L, Neilson GJ, et al. Lens cell survival after exposure to stress in the closed capsular bag. Invest Ophthalmol Vis Sci 2007; 48(6): 2701–2707.
23. Rabsilber TM, Limberger IJ, Reuland AJ, et al. Long-term results of sealed capsule irrigation using distilled water to prevent posterior capsule opacification: a prospective clinical randomised trial. Br J Ophthalmol 2007; 91(7): 912–915.
24. Sachdev GS, Soundarya B, Ramamurthy S, et al. Impact of anterior capsular polishing on capsule opacification rate in eyes undergoing femtosecond laser-assisted cataract surgery. Indian J Ophthalmol 2020; 68(5):780–785.
25. Rękas M, Kluś A, Kosatka M. Sealed-capsule irrigation with distilled deionized water to prevent posterior capsule opacification--prospective, randomized clinical trial. Curr Eye Res 2013; 38(3): 363–370.
26. Liu Z, Wang R, Lin H, et al. Lens regeneration in humans: using regenerative potential for tissue repairing. Ann Transl Med 2020; 8(22): 1544.

Figures

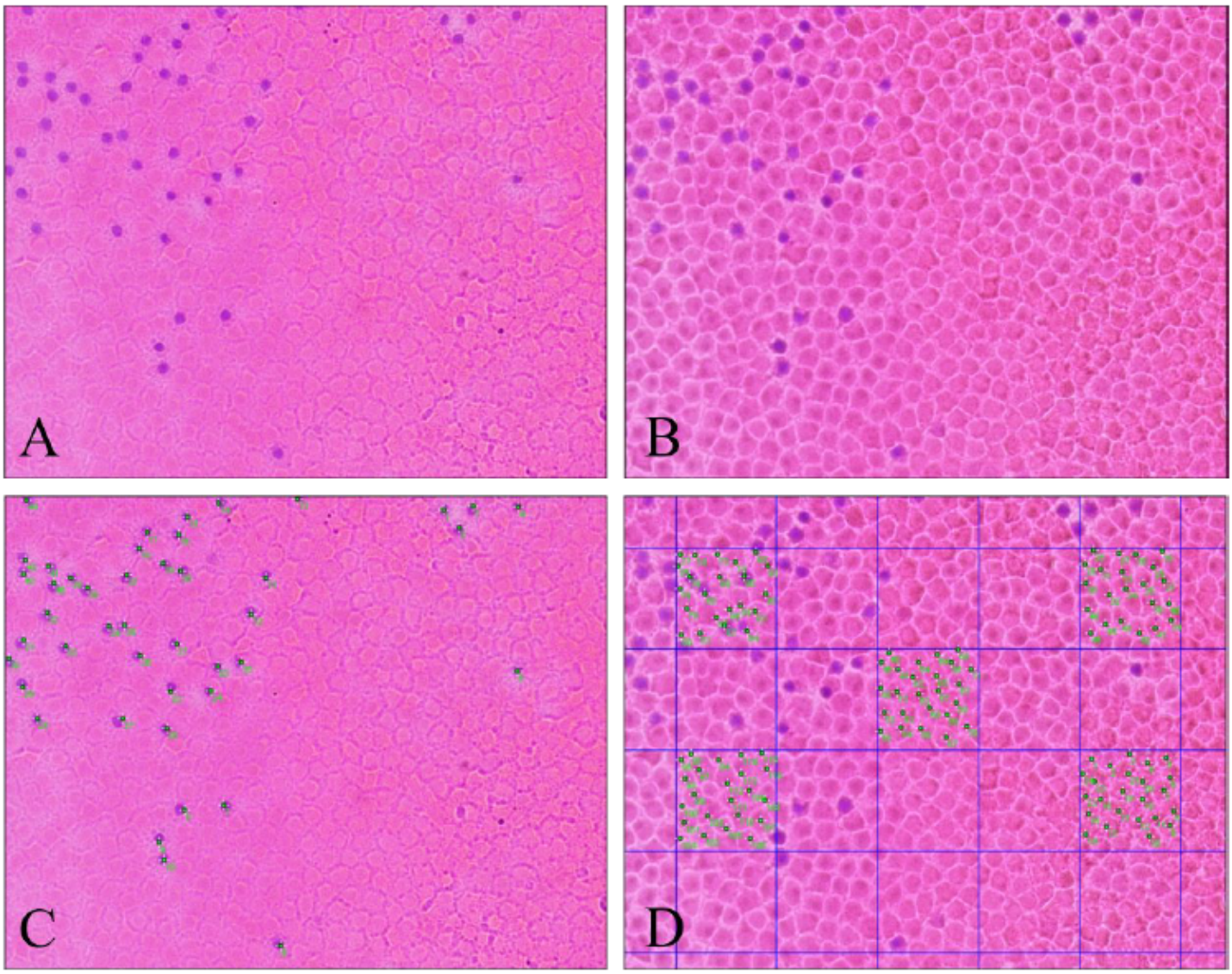

Figure 1

A schematic diagram of the detailed calculation procedures of the LECs density, the number of LECs deaths, and the LECs mortality. Taking photos at the nuclear level (A), the number of dead cells at the nuclear level, meaning the number of blue nuclei, was calculated. And then, taking photos at cell contour level (B), selecting 5 squares and calculating the number of LECs in them (per square: 0.005 mm2) (D). If the nuclei or cells were located at the boundary of the image, the principle of counting up but not down, and counting left but right was taken (C & D).

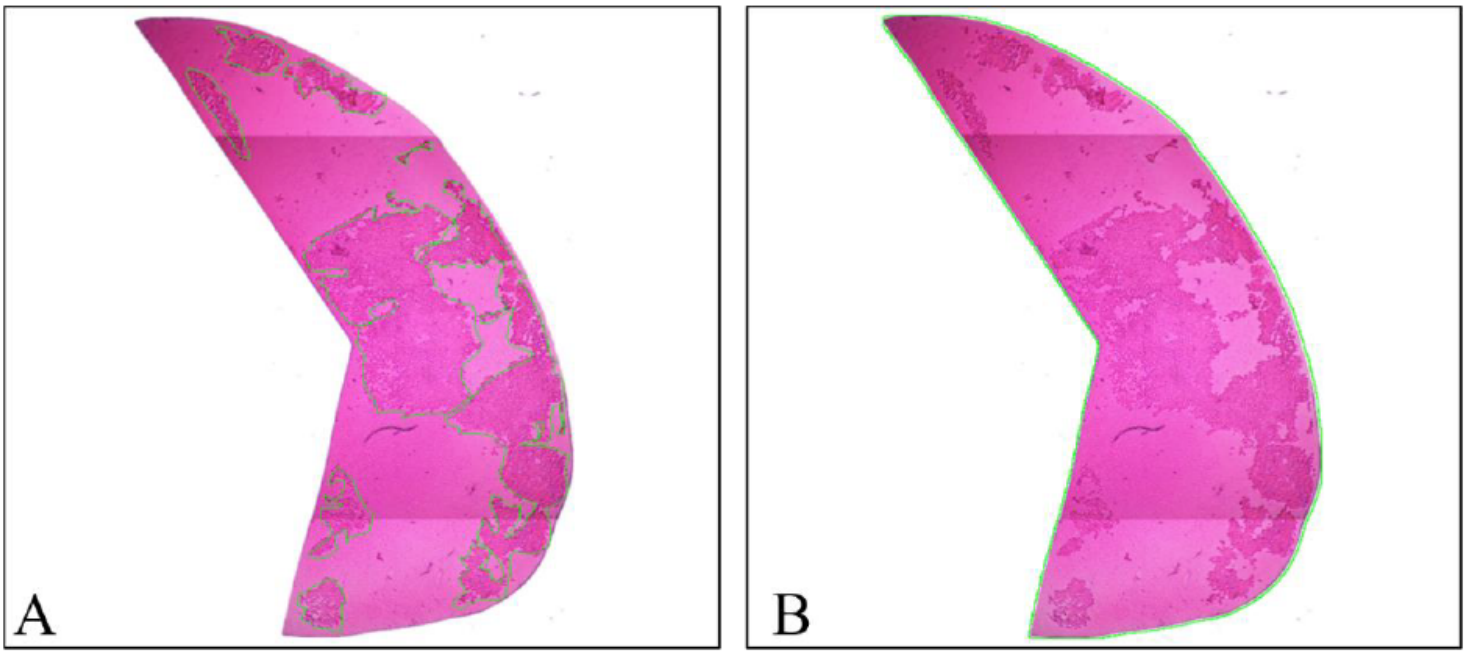

Figure 2

Specific procedure of calculating LECs shedding percentage by Image J. The LECs area and spliced capsule were circled and calculated three times, respectively, and the average values were taken (A & B).

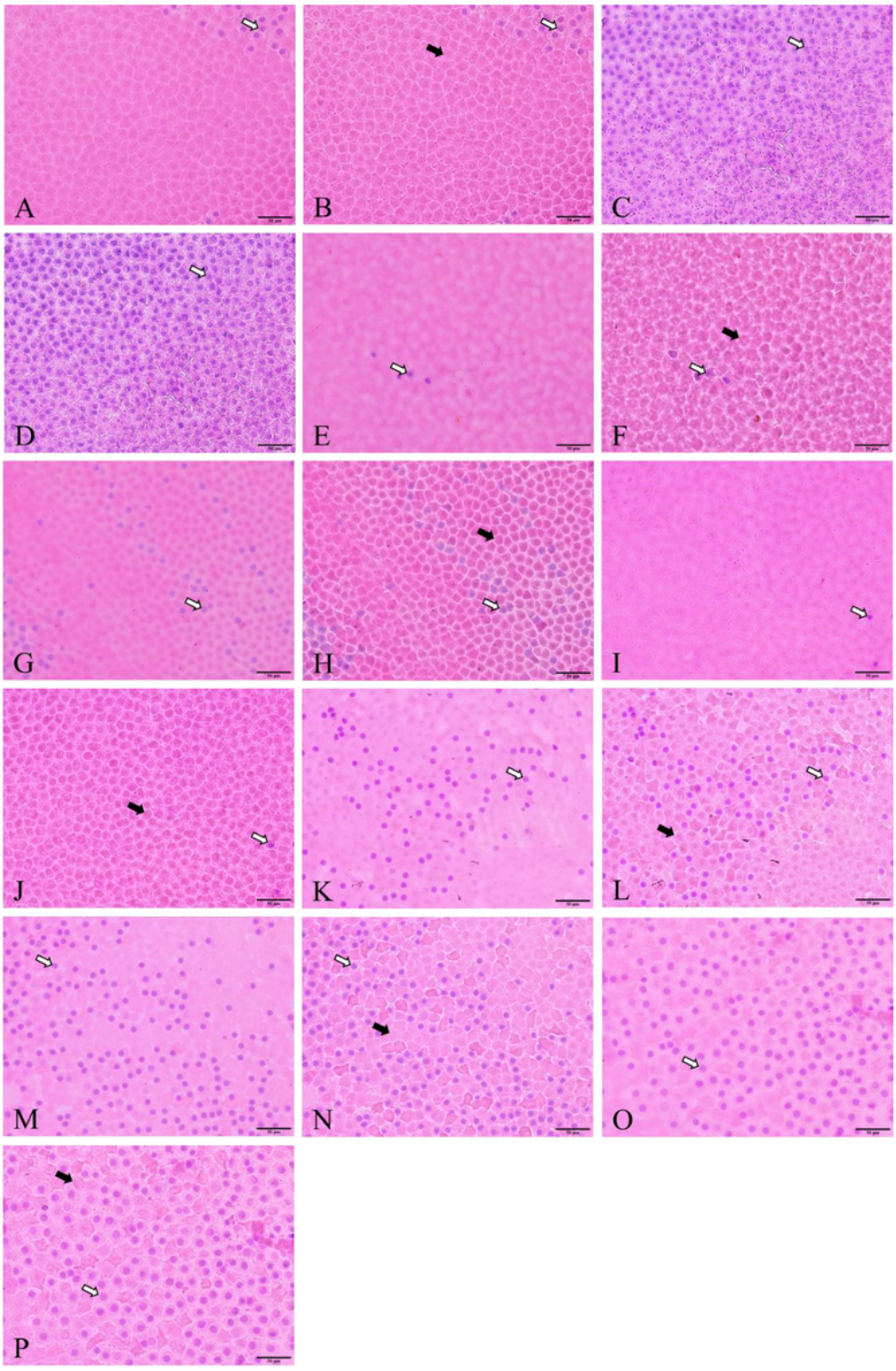

Figure 3

Lens epithelial cells (LECs) viability of the negative control group (A & B), positive control group (C & D) BSS group for 1, 2, and 3 mins (E & F, G & H, I & J), and DW group for 1, 2, and 3 mins (K & L, M & N, O & P). Photos were taken by light microscope at 400× after trypan blue-eosin staining. Half of the pictures (A, C, E, G, I, K, M, and O) were at nuclear level and the others were at cell contour level. (scale bar = 50 μm)

The white arrow represented the same dead cell of the same group at nuclear level or cell contour level. The black arrow represented the living cell at the cell contour level.

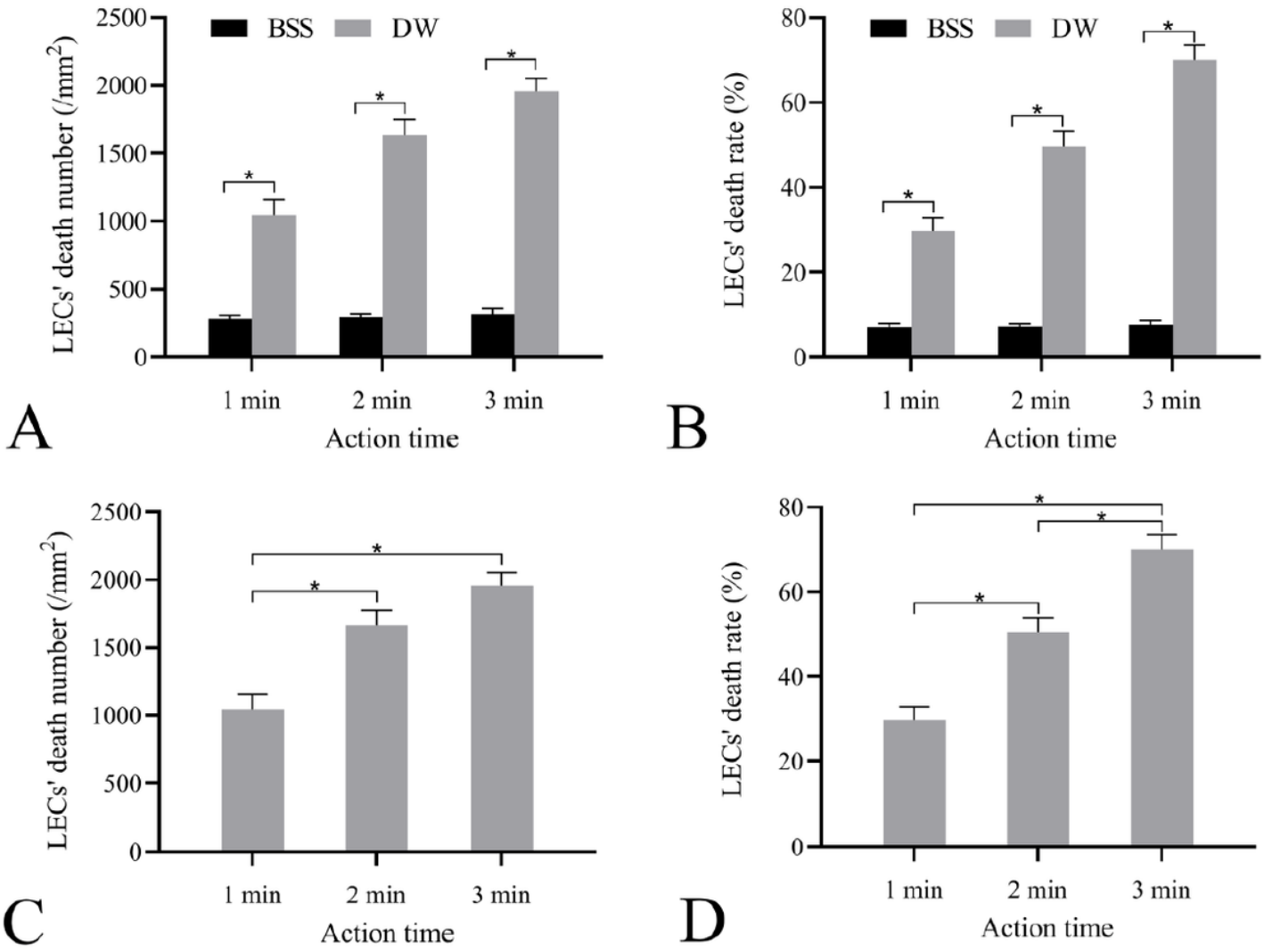

Figure 4

Effect of DW or BSS on LECs death number and LECs mortality. For the same soaking time, effect of DW or BSS on LECs death number (A) and LECs mortality (B). With different acting time, the effect of DW on LECs death number (C) and LECs mortality (D).

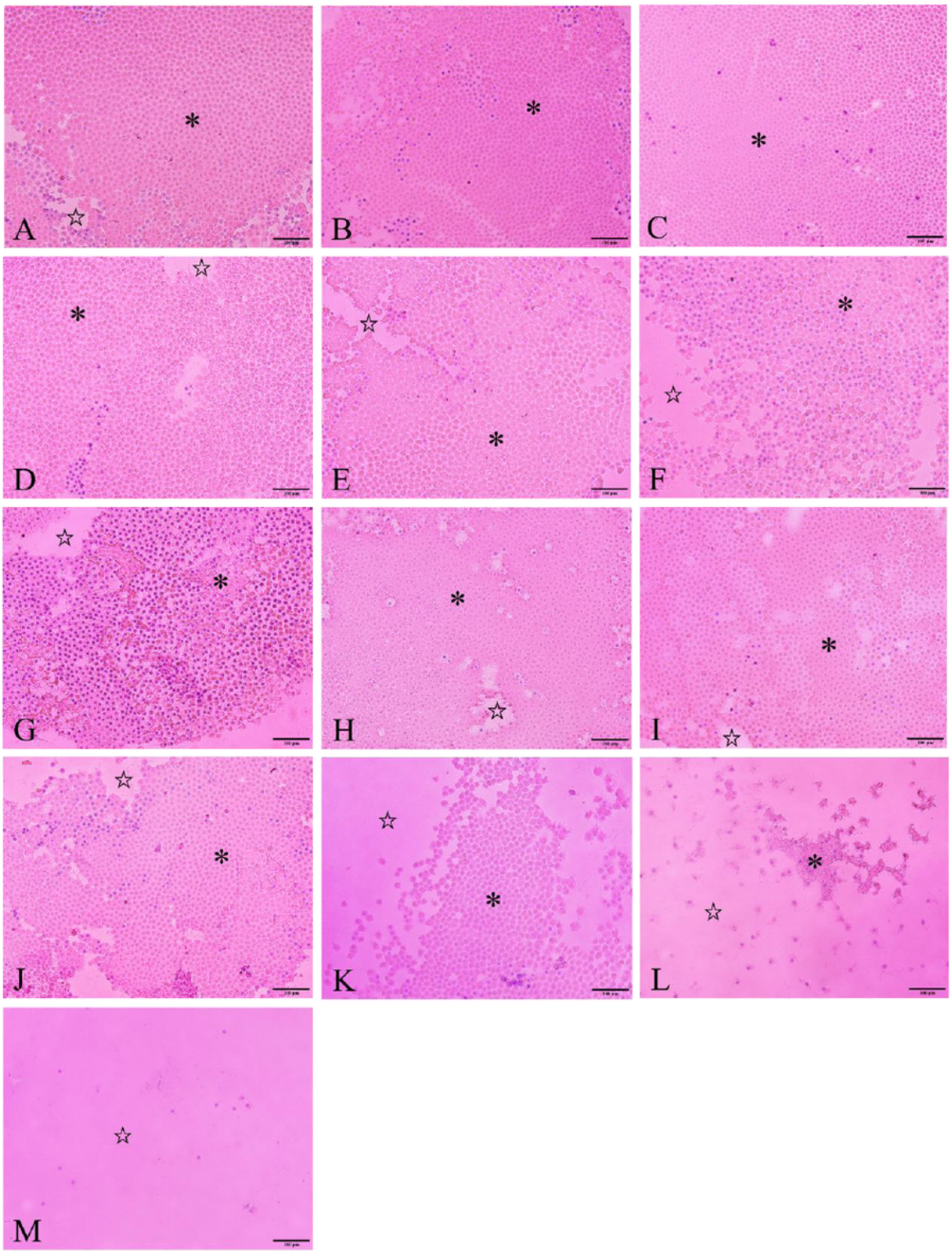

Figure 5

The morphology of the shed LECs in the negative control group (A), BSS group for 1, 2, and 3 mins (B, C, D), DW group for 1, 2, and 3 mins (E, F, G), BSS immersion for 1, 2, and 3 mins combined with rinse (H, I, J), and DW immersion for 1, 2, and 3 mins combined with rinse (K, L, M). An asterisk (*) represented regions with cells and a star (☆) represented regions without cells. Cells were stained with trypan blue-eosin, scale bar = 100 μm.

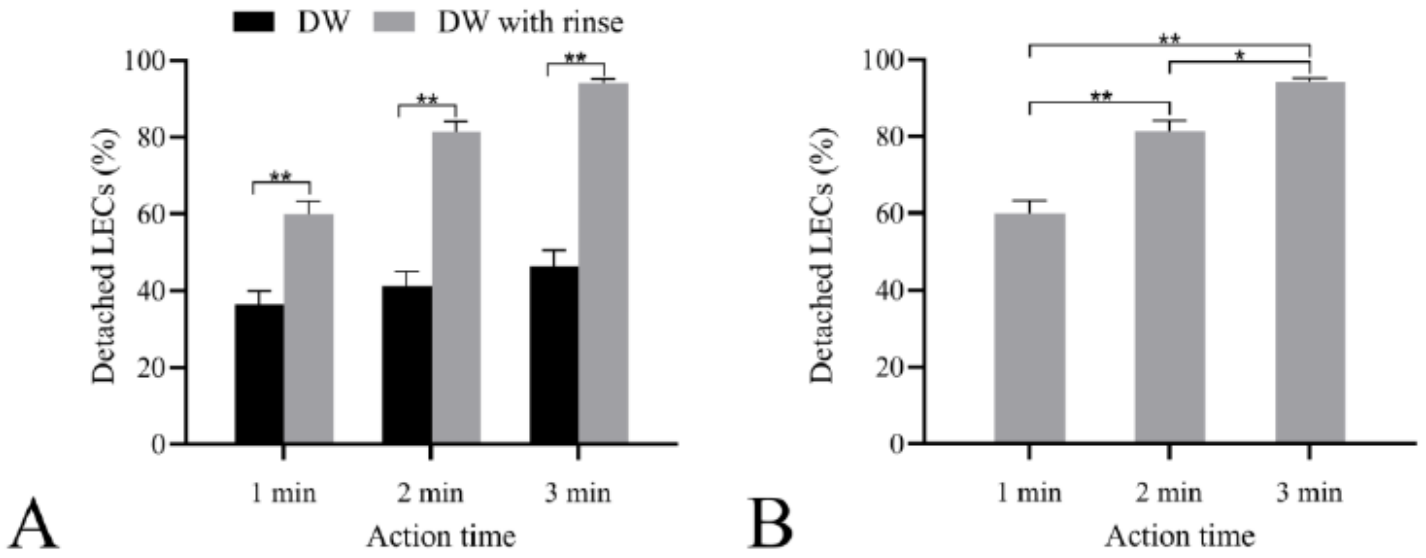

Figure 6

Effect of DW on the percentage of LECs shedding. A: With the same soaking time, the effect of DW with or without rinse on the percentage of LECs shedding. B: With the different duration time, the effect of DW with rinse on the percentage of LECs shedding. A single asterisk (*) represented the p values were less than 0.005 and a double asterisk (**) indicated p values were less than 0.001.

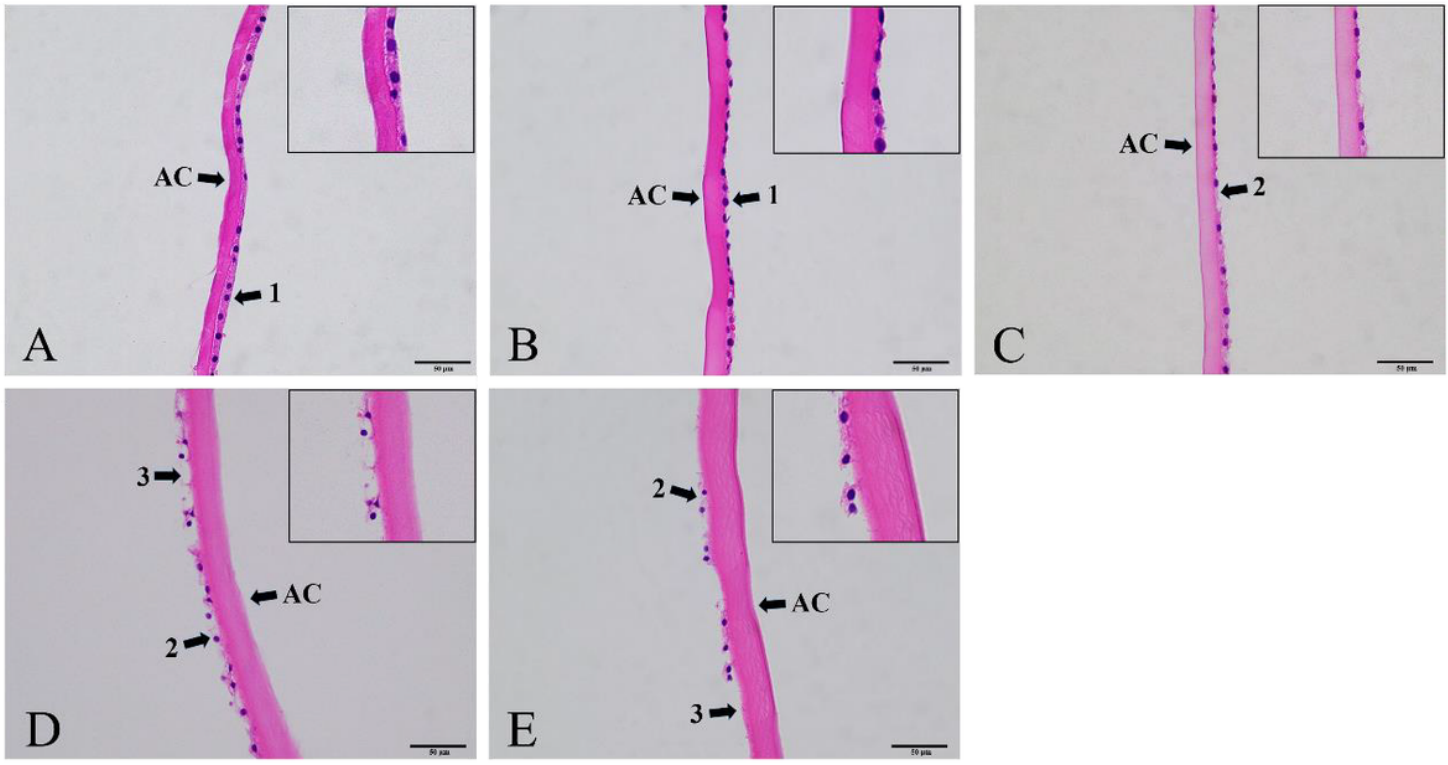

Figure 7

Microstructure of LECs in the negative control group (A), BSS group for 3 mins (B), DW group for 1 min (C), DW group for 2 mins (D), DW group for 3 mins (E). HE staining of the anterior capsule samples showed that the cell destruction in the DW subgroup for 1-, 2-, and 3-minutes, and LECs was partially exfoliated in the DW subgroup for 2-, and 3-minutes. (hematoxylin and eosin staining, scale bar=50 μm) 1: The cell showed a regular cell shape, a round nucleus, and a homogeneous cytoplasm. 2: This cell was with cytoplasmic destruction. 3: Shown here was cell shedding. AC: anterior capsule

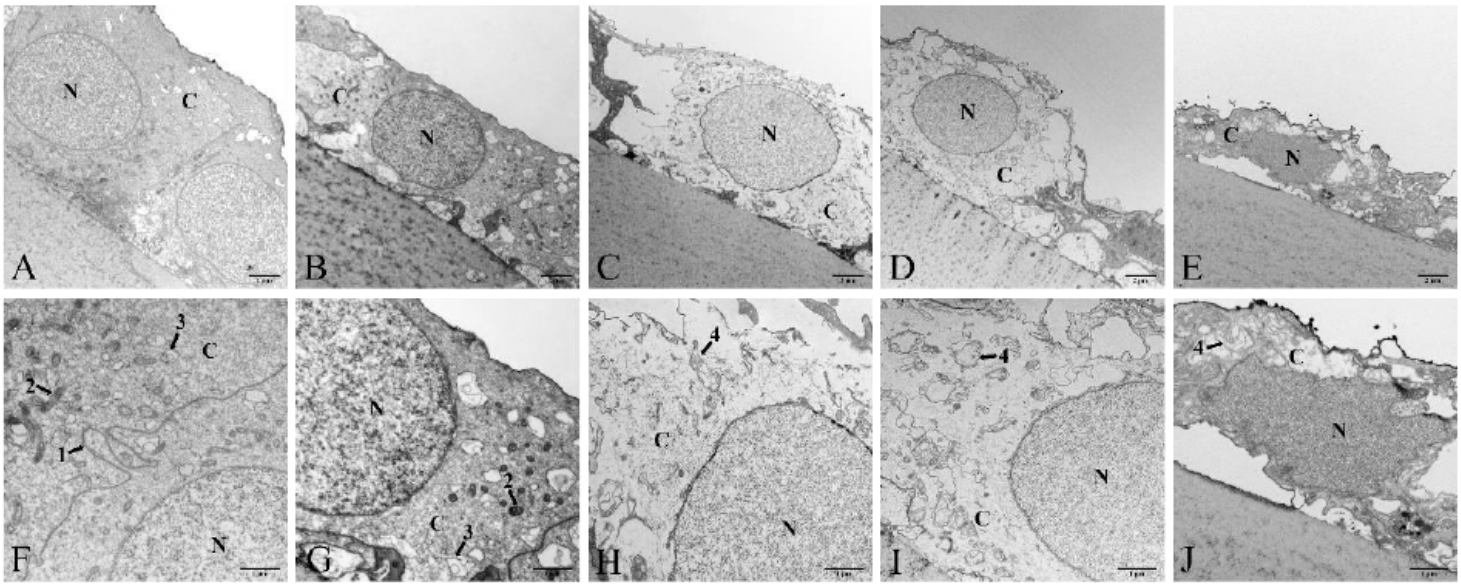

Figure 8

Ultrastructure of LECs in the negative control group (A & B), BSS group for 3 mins (C & D), DW group for 1 min (E & F), DW group for 2 mins (G & H), DW group for 3 mins (I & J). The TEM results showed that cytolytic destruction of LECs, swelling of organelles, and disruption of intercellular junctions in the DW subgroup for 1, 2 and 3 minutes. The cell-to-capsule junctions were loose in the DW subgroup for 1 and 2 minutes, and the cells were partially detached from the capsule in the DW 3 minutes subgroup. Pictures A, C, E, G, and I were under transmission electron microscope (TEM) at 8000×, and the scale bar was 2 μm. Other pictures were under TEM at 20000×, and the scale bar was 1 μm. 1: normal finger-like intercellular connectivity of LECs 2: normal mitochondria 3: normal lysosome 4: swelling of organelles N: nucleus C: cytoplasm