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Viability of Human Periodontal Ligament Fibroblasts in Tissue Culture After Exposure to Different Contact Lens Solutions

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Abstract

Aim: The viability of the periodontal ligament (PDL) cells is critical for successful healing of replanted avulsed teeth. Viability is primarily dependent on the duration of the extra-alveolar time and storage medium used to preserve teeth. Several storage media have been suggested but milk ranks highest. It would be desirable to evaluate other media as a suitable alternative for milk. The purpose of this study was to determine the viability of human PDL fibroblasts and their morphology after storage in different types of contact lens solutions.

Methods and Materials: PDL fibroblasts were cultured from a healthy extracted impacted human tooth and exposed to Bausch and Lomb (Renu), Ciba Vision (Titmus), and Alcon (Opti-free) contact lens solutions. Eagle's minimal essential medium served as control. The experiment was performed in plastic tissue culture clusters containing 24 wells. The PDL fibroblasts were grown in each well for three days. On the day of the experiment the culture medium was decanted, the cells were washed with phosphate buffered saline solution (PBS), and 1 ml of the tested solution was placed in each culture well. All tissue culture clusters were incubated at 37ºC in 5% CO_2 and 95% air for one, four, and 24 hrs. At the end of the incubation period, the cells were fixed and prepared for scanning electron microscope (SEM) examination.

Results: The results indicated Renu and Opti-free solutions were superior to Titmus solution in terms of their capacity to maintain the viability and normal morphology of PDL fibroblasts.

Conclusion: Contact lens solution is a good storage medium to maintain the viability of PDL fibroblasts for a short-term period.

Keywords: Periodontal ligament fibroblasts, contact lens solution, storage medium

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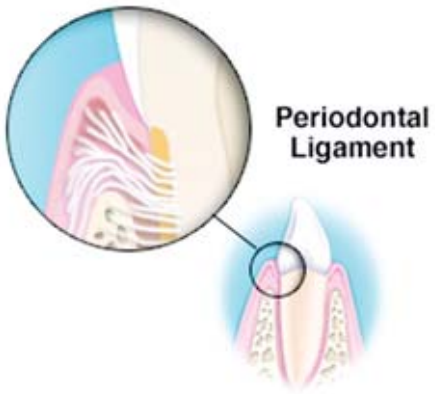
Introduction

Trauma to the teeth of both permanent and deciduous dentition is a very real problem facing dental practitioners. Injuries could occur to both hard and soft dental tissue including the pulp and the periodontium. Injuries to the periodontal ligament (PDL) can result in tooth avulsion. The viability of the PDL fibroblasts is critical for successful healing of replanted avulsed teeth.[1-3] The highest percentage of success can be achieved by immediate replantation or replantation within 30 minutes of avulsion.[4] Since this is not always possible, various temporary storage media have been recommended to prevent dehydration of the root surface, retain viability of the PDL cells, and to allow short-term storage of the avulsed tooth prior to replantation. Such media include saline, milk, Eagle's minimum essential medium, and Hanks balanced salt solution.

A wide variety of soaking solutions are presently available for contact lens care. These care products contain various antimicrobial preservative drugs such as polyaminopropyl biquanide, hydrogen peroxide, and polyquaternium. These preservatives have been proven to act as antimicrobial and anti-fungal agents to reduce the possibility of contamination during lens storage without detectable uptake by the lenses. Furthermore, they cause no ocular irritation. However, little is known about whether contact lens solutions are suitable temporary storage media for avulsed teeth in order to maintain the viability of PDL fibroblasts.[5]

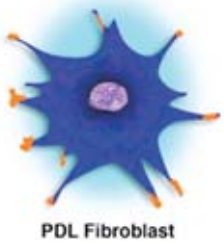
The purpose of this study was to determine the viability and morphology of human PDL fibroblasts after storage in different types of contact lens solutions using a scanning electron microscope (SEM).



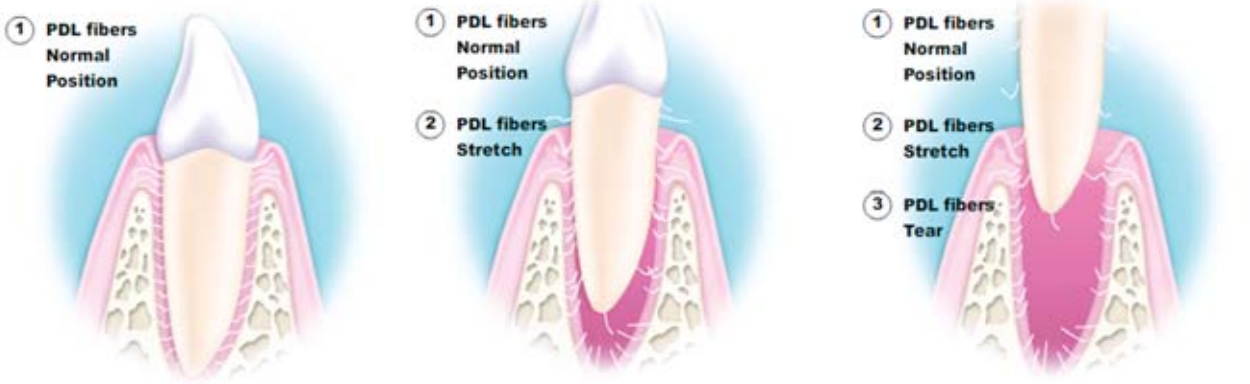
Methods and Materials

Periodontal Ligament Fibroblasts

Three-to-five day old cultures of human PDL fibroblasts were used in the study. The cells were obtained from a clinically healthy impacted upper third molar. The isolation and preparation of the primary cell cultures were previously described by Al-Nazhan.[6]


PDL Fibroblast

The cells were suspended in culture medium at a density of 5 x 10^5 cells / ml and were grown in a tissue culture flask (Coaster, Cambridge, MA, USA). The flasks were incubated at 37ºC in 5% CO_2 and 95% air. During growth the medium was changed every other day and the day before performing the experiment.



Culture Medium

Eagles MEM with Earle's BSS supplemented with 10% (v/v) fetal calf serum, 2m ML- glutamine, and 2.2 mg sodium bicarbo nate per ml was used as the culture medium. In addition 100 I.U / ml penicillin, 100 mg / ml erythromycin, and 2.5 mg /ml fungi zone were added to the culture medium (Sigma Chemical, St. Louis, MO, USA).

Storage Medium

As shown in Figure 1, the following storage media were used for this experiment:

Figure 1. Storage media used in the study.

Renu contact lens solution (Group 1)

Renu Multifunction™ solution composed of 1% poloxamine, which is a surface active agent that cleans and prevents debris deposits; 0.00005% polyaminopropyl biquanide (Dymed)® as a preservative; and sterile buffered saline solution (Bausch and Lomb, Inc. Rochester, NY, USA).

Titmus contact lens solution (Group 2)

Titmus solution composed of 0.06% H_2O_2 with no preservative (Ciba Vision Corporation, Duluth, GA, USA).

Opti–free contact lens solution (Group 3)

Opti–free lens solution had a content of 0.05% edetate disodium and 0.001% polyquaternium (Polyquad)® (Opti–free Alcon Laboratories Inc, Fort Worth, TX, USA).

Experimental Procedure (Figure 2)

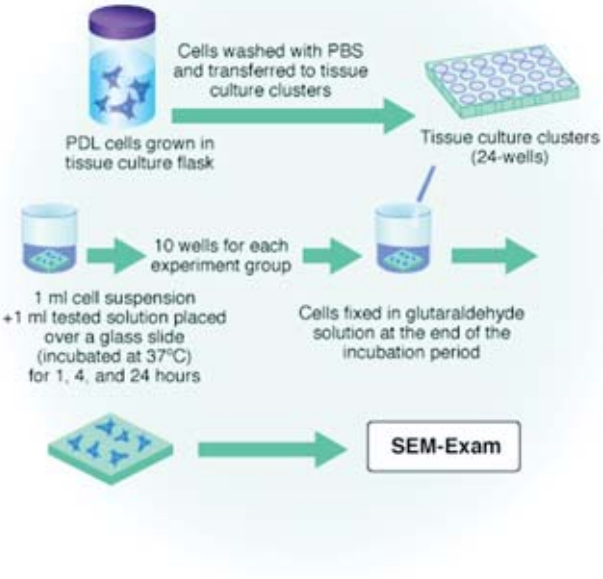


Figure 2. Storage media used in the study.

The culture medium was drained from each tissue culture flask, and the cells were washed three times with phosphate buffered saline solution (PBS). Cells were then harvested with 0.02% trypsin and washed in PBS before being suspended in culture medium. The experiment was performed in plastic tissue culture clusters (Falcon, Becton Dickinson Co., Franklin Lakes, NJ, USA) containing 24 wells each with an inner diameter of 16 mm. A sterilized 5mm^2 glass slide was placed in the bottom of each culture well, and a small scratch was made on the corner of each glass slide for an easy identification and orientation of the sample during SEM examination. One millimeter of cell suspension mixed with 1 mm of culture medium was placed in each well. All tissue culture clusters were then incubated at 37ºC in 5% CO_2 and 95% air for 24 hr to allow the cells to attach to the glass slide. The cells were examined under a light microscope. The medium was decanted and the cells were rinsed three times with PBS solution. Fresh culture medium was then placed in each culture well and the cells were allowed to grow for three days.

On the day of the experiment the culture medium was decanted and the cells were washed with PBS solution. The tissue culture clusters containing the attached PDL cells were divided into four experimental groups. One millimeter of each tested solution was placed in an individual well without culture medium. At the same time, 1 mm of culture medium was added to culture wells with no tested solutions to serve as control. All the culture wells were incubated at 37°C and 100% humidity for one, four, and 24 hours. A total of 105 culture wells were used. For each observation period, five wells were used for control and ten wells were used for each experimental group.

Microscopic Sample Preparation

At the end of the incubation periods, the cells were prefixed in 0.1% glutaraldehyde in tested solution for five minutes. The solution was then decanted and replaced with 2.0% glutaraldehyde in 100 mM Na–cacodylate buffer (PH = 7.2) at room temperature and fixed for half an hour in the same solution. Specimens were dehydrated (five minutes each) through 50%, 70%, 90%, and 100% ethanol. The glass slides were carefully removed and transferred for drying with CO_2 using a Samdri PVT 3 –BG machine (Tausimis, Rockville, MD, USA). The glass slides were mounted with silver conducting paint and gold–sputter coated to a thickness of 5–7 nm. Specimens were then examined with a Joel JSM–T330 SEM (Peabody, MA, USA), operated at an accelerating voltage of 25 Kv.

Results

Normal Morphology

Figure 3 illustrates the normal morphologic feature of the PDL fibroblasts.

One-Hour Exposure Observation

After one hour of incubation, numerous discoid shape cells with smooth surfaces were seen in the control wells. Few elongated cells were occasionally seen with small blebs and microvilli covering the cell surface. Lamellopodia attachment was also observed. No dividing cells were seen.

The one-hour exposure of the PDL fibroblasts to the test solutions showed normal morphology. Many cells appeared spindle or elongated with ruffles and covered the cell surface (Figure 4). The cells were attached to the substrate with lamellopodia and filopodia.

Four-Hour Exposure Observation

The cells in the control wells were round, flattened, or stellate in shape after four hours of incubation. The cell surface was smooth or covered with ruffles. The cells attached to the substrate with lamellopodia. No dividing cells were seen.

The four-hour exposure of the PDL fibroblasts to the test solutions showed normal morphology. In general, the cells appeared elongated and spindle in shape (Figure 5). Cells were covered with ruffles and small blebs. Cells attached to the substrate with lamellopodia and filopodia. Many dividing cells were seen throughout the culture. Exposure of the cells to Titmus showed a few roughly spindle shaped and rounded cells with irregular surfaces covered with blebs and tissue debris (Figure 6).

Twenty-Four Hours Observation

When the experimental time was increased to twenty-four hours, the cells in the control wells showed well-developed cells. Fully spread, elongated, spindle or fan-like shaped cells were seen (Figure 7). The cells have a smooth surface and few microvilli. The cells attached to the substrate with lamellopodia and filopodia.

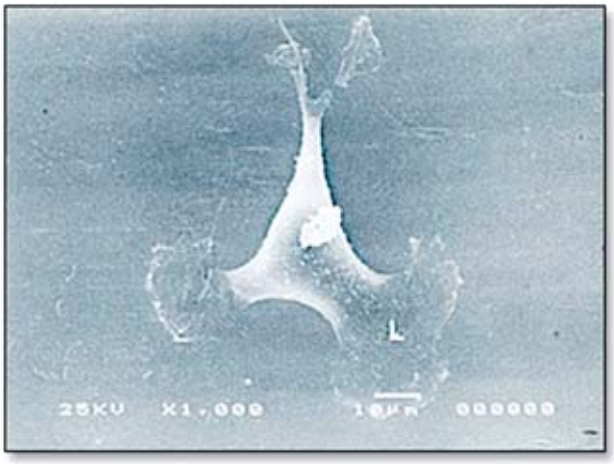


Figure 3. SEM of a PDL fibroblast attached to the glass slide at 3-5 days observation. Cell is fan-like shape with small blebs on its body and attached to the glass with lamellopodia.

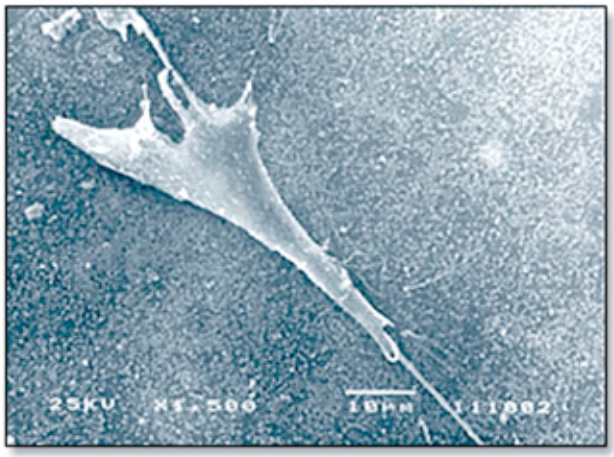


Figure 4. SEM of PDL fibroblasts attached to the glass slide at 1 hr observation after storage in Renu solution. Elongated, fan-like shaped fibroblast with smooth surface and few ruffles covering the cell surface.

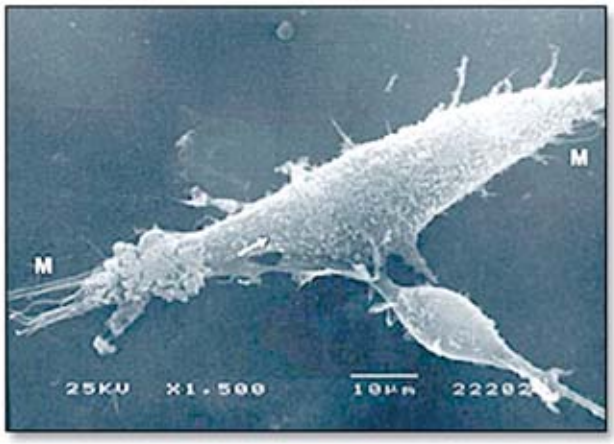


Figure 5. SEM of PDL fibroblasts attached to the glass slide at 4 hours observation after storage in Renu solution. Spindle shaped cells covered with ruffles (arrow) and attached to the substrate with lamellopodia and microvilli.

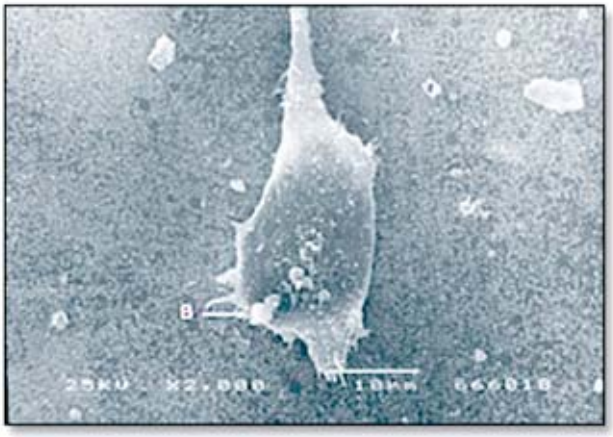


Figure 6. SEM of PDL fibroblasts attached to the glass slide at 4 hours observation after storage in Titmus solution. Cell is roughly spindle with large blebs on its surface.

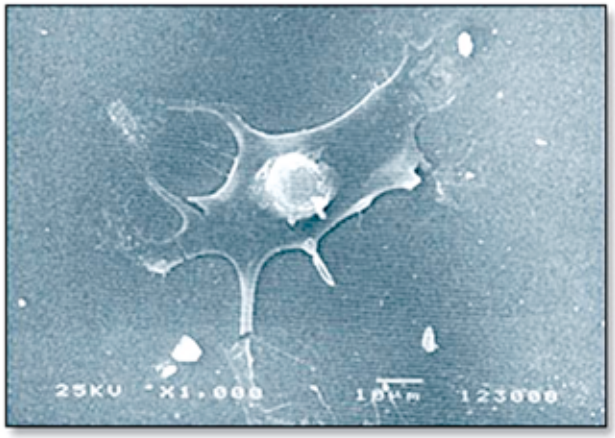


Figure 7. SEM of PDL fibroblasts attached to the glass slide at 24 hours observation. A fully spread cell with smooth surface and attached to the substrate with lamellopodia.

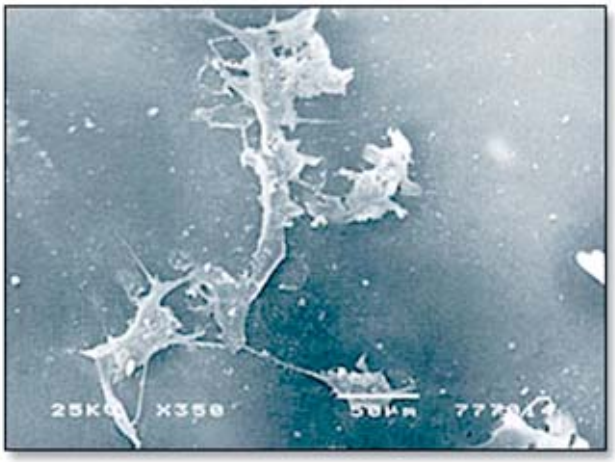


Figure 8. SEM of PDL fibroblasts attached to the glass slide at 24 hours observation after storage in Titmus solution. Remnants of tissue of damaged cells.

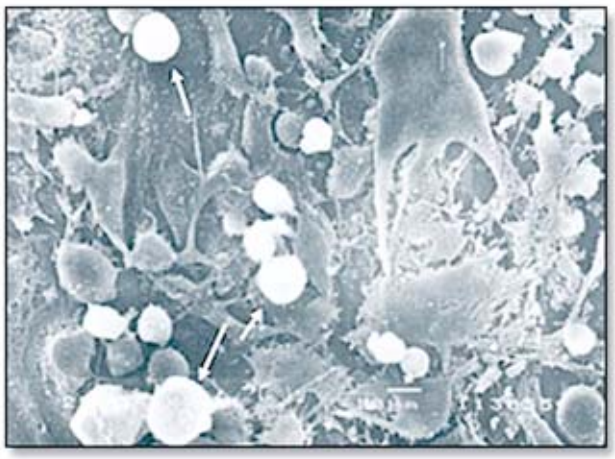


Figure 9. SEM of PDL fibroblasts attached to the glass slide at 24 hours observation after storage in Opti-free solution. Dividing round cells (arrow) covered with microvilli and stellate shaped cells with smooth surface are seen.

The 24 hr exposure of the PDL fibroblasts to the test solutions showed normal morphology except for the Titmus solution (Figure 8). Cells were elongated, spindle, and stellate in shape, and the cell surface was smooth or covered with ruffles. The cells attached to the substrate by lamellopodia and filopodia. Dividing round cells covered with microvilli were only seen in the wells of the Opti-free solution (Figure 9).

Discussion

In the present study the viability of PDL fibroblasts was determined *in vitro* after exposing them to various contact lens solutions used as storage media for different observation periods. The procedure and culturing condition and storage of PDL fibroblasts was easy and suitable. There are principally two main ways of harvesting cells from PDL tissue for tissue culture studies. One technique involves the use of certain enzymes such as trypsin for the release of cells from the tissues. In the other method the cells are allowed to grow out from a tissue biopsy. Fibroblasts have been established often by trypsinization of human teeth but no epithelial-like cell cultures have been obtained.[7] Trypsinization of the PDL tissue was done in this study, and the general morphology of the cells observed by SEM showed no epithelial-like cells. The PDL fibroblasts grow to a confluent monolayer, and their morphology was similar to previously reported studies.[5,8-10] Their growth characteristics were identical and similar to fibroblasts cultivated from other species.[11] Fibroblasts are usually the predominant cells obtained from the cultured PDL tissue. According to Gilbert and Migeon[12] the reason for fibroblasts taking over mixed culture is not understood. In the present investigation the fourth subculture of the PDL fibroblasts was used. It proved to be practical and relatively free of tissue remnants. At this time, minimal alteration of cell morphology and function due to cultivation will have occurred.

The light microscope has been used extensively by several investigators to examine morphological cell changes.[5,13] However, the use of a light microscope lacks fine details. The use of SEM in this study was very useful for investigating the general shape and surface morphology of the cells. Furthermore, the specimen preparation is comparatively easy and the resolution and depth of focus is greatly increased. In addition, it

provides high magnification and three-dimensional perspective of surface morphology.[14-16] Thus, a change in cellular morphology is a particularly valid criterion for evaluation of cell damage.

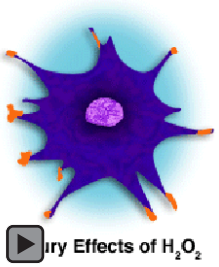
The experiment was performed in a tissue culture cluster where direct contact between the cells and the tested (storage) solution is achieved. The method used was similar to other studies.[5,9,17] The glass surface used for fibroblasts growth has been reported to be a good substratum for normal cell growth *in vitro*.[8,17]

The initial attachments of the cells to the substrate occurred after one hour of cell suspension in culture media. However, the control culture PDL fibroblasts at one, four, and 24 hours exhibited normal morphology. This observation is in agreement with other studies.[8,15,18] As cell spreading proceeds, the cells can assume a variety of shapes. The degree of spreading cells varied from one cell to another. Some of the PDL fibroblasts exhibited normal morphology, while others did not. This may be attributed to the fact the cells have different stages of growth that might behave differently.[19]

Contact lens solutions are commonly available in pharmacies. They are basically sterile saline solutions with some preservative and used as rinsing and storing solution. Hung et al.[5] evaluated contact lens solutions including Opti-free as storage media of human PDL fibroblasts in tissue culture and compared them to saline and milk. The cells were stored in the storage media for up to 96 hours. The morphological cell changes were examined through an invested phase contrast microscope. Their findings showed the contact lens solution gave a poor result compared to milk. They reported very few healthy cells remained attached to the substrate after four hours where most of the cells were rounded and shriveled. This contradicts with the findings of the present study. The PDL fibroblasts in the present study remained viable and showed normal morphology through all observation periods. Huang et al.[5] did count the cells using a phase contrast microscope and divided the cells into healthy cells with spindle shape and unhealthy cells having round shape. The round cells reported could be dividing cells. The round cells seen in the present study using a SEM were covered with microvilli and this is important for cell spreading.

Opti–free contact lens solution has been shown to be good storage media for maintaining PDL fibroblasts viability in all observation periods. The result of this study was in agreement with the previous findings.[20] In addition, Sigalas et al.[20] tested the ability of cells to survive and recovered at room temperature from exposure to different contact lens solutions including Opti-free, milk, culture medium, Hanks balanced salt solution, tap water, and Gatorade. They reported the Opti-free contact lens solution is superior to milk. Morgan et al.[21] found polyquad, which is one of the main ingredients of Opti-free solution, had a lower level of toxicity and was not associated with typical patient sensitivity problems up to three months.

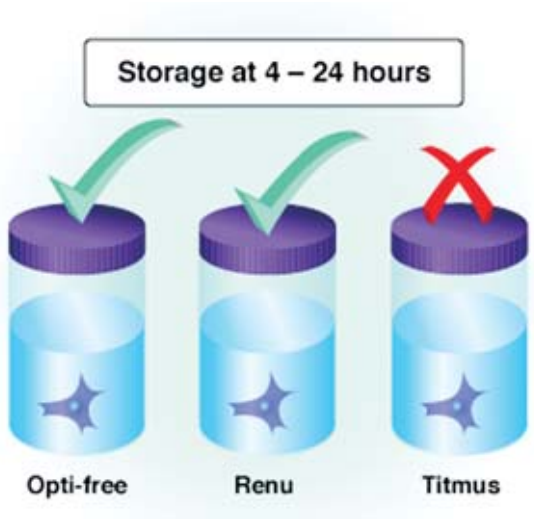
Storing PDL fibroblasts in Titmus solution is time dependent. Damages of PDL fibroblasts had increased markedly between four and 24 hours of storage. This could be related to the H_2O_2 content of the Titmus solution. Tripathi and Tripathi[22] reported morphologic alterations in cultured human corneal epithelial cells with 0.06 H_2O_2.This could explain the oxidative injury effects of H_2O_2.



Dividing cells were observed mainly in Opti-free and Renu solutions. The presence of the dividing cells means the storage solution provided a good nutrient or it did not harm the cells.

Conclusions

The morphological findings of this study showed contact lens solution is a good storage medium for human PDL cells. We recommended further investigations using SEMs to study the effect on the intercellular structures of the tested cells.



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