Milk and egg albumen are superior to human saliva in preserving human skin fibroblasts

Nathan Rozenfarb, DDS, MS Ari Kupietzky, DMD, MSc Zia Shey, DMD, MS

Abstract

The purpose of this in vitro study was to compare egg albumen, whole bovine milk, human saliva, and tissue culture medium (MEM) for effect on the viability of human skin fibroblasts and their osmolalities. Confluent monolayers of fibroblasts were grown. Growth medium was poured off and dishes were divided into five groups, 15 dishes each of: 1) chick egg albumen; 2) fresh whole milk; 3) human saliva; 4) tissue culture medium; and 5) bench-dried storage without any media. After 15, 45, and 90 min the average number of vital cells was measured using the trypan blue dye exclusion test. Tissue culture medium represented the best preservation media for human skin fibroblast cells (92.8% at 45 min, 87.6% at 90 min). No significant differences were observed between milk and albumen, with a majority of the cells surviving after 90 min (67.6% and 70.2%, respectively). Human saliva, due to its hypotonicity, markedly swelled the cells, causing decreased cell viability (27.4% at 90 min). Bench-dried cells, as expected, showed no viable cells as early as 15 min. The osmolality of the MEM, milk and egg albumen ranged between 251–298 mOsm/kg, whereas the saliva was hypotonic, with an osmolality of 73 mOsm/kg. (Pediatr Dent 19:347–48, 1997)

Preservation of the remaining periodontal ligament cells on an avulsed tooth is of utmost importance to the successful outcome of replantation. The viability of these cells depends on the time elapsed between avulsion and replantation and on the storage medium in which the tooth is placed.[1] A suitable medium must be readily available and able to preserve the tissues until treatment can be rendered. Studies suggest that bovine milk is a suitable storage medium.[2–4] However, one suitable medium may not be available and alternatives for bovine milk are needed. Commercially prepared storage media (e.g., Hank's™ balanced solution, Viaspan™ or Eagle's™ medium) have been shown to be effective[5], but their availability near the site of an accident is doubtful. Products most commonly found at home are more practical and should be evaluated for effectiveness in preserving tissues on an avulsed tooth. Chick egg albumen is a sterile solution readily available in most households and many schools.

The purpose of this study was to compare egg albumen, milk, human saliva, and tissue culture medium for effect on the viability of human skin fibroblasts in vitro and their osmolalities.

Materials and methods

Human skin fibroblasts obtained from the cytogenics laboratory of the New Jersey Medical School were plated in 25 cm^2 Nunc™ multidish flat bottom wells and grown at 37°C in Eagle's™ Minimum Essential Medium (MEM), supplemented with 4 mM glutamine, 10^5 IU/l penicillin, 100 μg/ml streptomycin, and 10% w/v fetal calf serum in a humidified incubator at 5% CO_2. The cells were allowed to grow until a minimum of 70% confluency was achieved. Growth medium was poured off and dishes were divided into five groups, 15 dishes each of: 1) chick egg albumen (separated from the yolk by pouring the albumen from one half of the shell into the other); 2) whole pasteurized bovine milk; 3) human mixed saliva (obtained from three individuals just before the experiment); 4) bench-dried storage (cells were allowed to dry at room temperature without any media); and 5) tissue culture medium (MEM). The cells were then treated identically with each experimental medium as follows: 3 ml of experimental medium was added to the monolayer for 15, 45, and 90 min being left uncovered under the sterile hood at room temperature. Each incubation period had five samples of each experimental medium. The bench-dried group was left uncovered at room temperature without any medium for the same time periods as above. Following the various incubation periods, the experimental media were removed and the cells washed three times with 3 ml phosphate buffered saline (PBS) followed by 1 ml of 0.05% trypan blue (w/v). After 5 min at room temperature, the solution was discarded, the cells washed again with 3 ml PBS, and examined microscopically with a Nikon™ inverted microscope. Cell viability was determined by observation of four fields of view. Each of the five experimental media was tested for osmolality by placing 2-ml samples in an automatic osmometer (Osmette A™, Waldham, MA). Each sample was tested five times. The

chi-square test was used for analysis and differences were considered significant at the 0.05 level.

Results

After 15 min, no differences were observed between fibroblast viability in tissue culture medium, chick egg albumen, or whole bovine milk ($P > 0.05$)(Table). Storage in any media did not significantly reduce the number of viable cells. However, cells stored in human saliva presented a significant decrease in cell viability (chi square = 5.4, $P < 0.05$). Bench-dried cells showed no viability after 15 min. After 45 min, cells stored in tissue culture medium still showed no significant decrease in cell viability, whereas chick egg albumen and whole bovine milk cells showed 74% and 80% viability, respectively. Following 90 min of storage, an insignificant reduction in cell viability was observed in tissue culture cells 87.6% (chi square = 3.7, $P > 0.05$). Cells stored in milk showed a significant reduction in cells as compared with cell viability in milk after 45 min of storage (67.6% versus 80%, chi-square = 9.1, $P < 0.05$). No further reduction in cell viability was observed in chick egg albumen when compared with 45 min storage (70.2% versus 74%, chi-square = 0.02, $P > 0.05$). The osmolality of the MEM, milk, and egg albumen ranged between 251–298 mOsm/kg, whereas the saliva was hypotonic, with an osmolality of 73 mOsm/kg.

TABLE. OSMOLALITY OF MEDIA AND VIABILITY* OF SKIN FIBROBLASTS IN THEM AT 15, 45, 90 MINUTES

Medium (osmolality)†	*15 minutes*	*45 minutes*	*90 minutes*
Tissue culture medium (298 ± 1)	98.6 ± 1.2	92.8 ± 1.9	87.6 ± 3.6
Chick egg albumen (251 ± 3)	81.4 ± 2.6	74 ± 3.7	70.2 ± 1.6
Whole bovine milk (268 ± 1)	86.8 ± 3.2	80.2 ± 4.4	67.6 ± 4.5
Pooled human saliva (73 ± 2)	66.2 ± 3.2	32.8 ± 2.8	27.4 ± 4.5
Bench-dried (NA)	No viable cells	No viable cells	No viable cells

* Mean values are represented in percentage of viable cells.

† mOsm/kg

Discussion

Previous studies have established the validity of using human fibroblasts as a substitute for PDL cells in in vitro interim cell media research.[5] When cultivating human PDL cells fibroblast-like cells predominate.[2] Many metabolic and morphologic similarities exist between human skin fibroblasts and PDL fibroblasts in vitro.[2,4] The results of this study were very similar to those of Blomlof and others who used PDL cells.[2,6]

Chick egg albumen was tested in this study for the first time. Results of this study reveal that albumen preserves fibroblasts at least to the same extent that milk does. The physiologic osmolality of chick egg albumen may be partially due to its high water content (87.8%), and levels of dextrose (0.4%), sodium chloride (0.3%), and nitrogen (2%), which compose its main proteins of ovalbumen, conalbumen, and ovomucin.[7] In contrast, the poor results observed with human saliva can be attributed to its low osmolality. Hypotonic solutions cause cell swelling, which stretches the cell membrane, and potentiates the effect of bacterial products and toxins present in saliva. Other suggested storage media include water, the vestibule of the mouth, physiologic saline, and cell culture media in specialized transport containers.[8] Water was not included in this study because it is the least desirable storage medium due to its hypotonic osmolality, which causes rapid cell lysis.[3] The best storage media for PDL cells are pH-balanced cell preserving solutions such as Hank's™ balanced solution, Viaspan™ or Eagle's™ medium.[8] However, their availability at home is doubtful. Albumen could be the most suitable sterile storage solution found in a household—a frequent scene of dental trauma.

Dr. Rozenfarb is the section head, Department of Pediatric Dentistry, at Poriya Hospital in Poriya, Israel. Dr. Kupietzky is in private practice in Jerusalem, Israel. Dr. Shey is a professor, Department of Pediatric Dentistry, and an associate dean for Student Affairs and Graduate Dental Education, at the New Jersey Medical School, Newark.

1. Krasner P, Rankow J: New philosophy for the treatment of avulsed teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 79:616–23, 1995.
2. Blomolf L: Milk and saliva as possible storage media for traumatically exarticulated teeth prior to replantation. Swed Dent J Suppl 8:1–25, 1981.
3. Blomolf L, Lindskog S, Anderson L, Hedstrom KG, Hammarstrom L: Storage of experimentally avulsed teeth in milk prior to replantation. J Dent Res 62:912–16, 1983.
4. Courts F, Mueller U, Tabeling H: Milk as an interim storage medium for avulsed teeth. Pediatr Dent 5:183–86, 1983.
5. Trope M, Hiltz J: Vitality of human lip fibroblasts in milk, Hanks™ balanced salt solution and Viaspan™ storage media. Endod Dent Traumatol 7:69–72, 1991.
6. Blomlof L, Otteskog P: Viability of human periodontal ligament cells after storage in milk or saliva. Scand J Dent Res 88:436–40, 1980.
7. Parkinson J: The chemical composition of eggs. J Sci Fd Agric 17:101–9, 1966.
8. Trope M: Clinical management of the avulsed tooth. Dent Clin North Am 39:93–112, 1995.