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

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Abstract

The purpose of this 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 hypertonicity, 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)
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). Stor-

age 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 vi-

ability 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 com-

pared 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 = 0.2, P > 0.05). The os-

molarity of the MEM, milk, and egg albumen ranged between 251-298 mOsm/kg, whereas the saliva was hypotonic, with an osmolarity of 73 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. When cultivating human PDL cells fibroblast-like cells predominate. Many metabolic and morphologic similarities exist between human skin fibroblasts and PDL fibroblasts in *in vitro*. The results of this study were very similar to those of Blomlof and others who used PDL cells.

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 pro-

teins of ovalbumen, conalbumen, and ovomucin. In con-

trast, the poor results observed with human saliva can be attributed to its low osmo-

lality. Hypotonic solutions cause cell swelling, which stretches the cell mem-

brane, and potentiates the effect of bacterial products and toxins present in sal-

iva. Other suggested storage media include water, the vestibule of the mouth, physiologic saline, and cell culture media in specialized transport containers.

Water was not included in this study because it is the least desirable storage medium due to its hypotonic osmo-

lality, which causes rapid cell lysis. The best storage media for PDL cells are pH-balanced cell preserving so-

lutions such as Hank's™ balanced solution, Viaspan™ or Eagle's™ medium. 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.

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cal School, Newark.

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

<table>
<thead>
<tr>
<th>Medium (osmolality)†</th>
<th>15 minutes</th>
<th>45 minutes</th>
<th>90 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue culture medium (296 ± 1)</td>
<td>98.6 ± 1.2</td>
<td>92.8 ± 1.9</td>
<td>87.6 ± 3.6</td>
</tr>
<tr>
<td>Chick egg albumen (251 ± 3)</td>
<td>81.4 ± 2.6</td>
<td>74 ± 3.7</td>
<td>70.2 ± 1.6</td>
</tr>
<tr>
<td>Whole bovine milk (268 ± 1)</td>
<td>86.8 ± 3.2</td>
<td>80.2 ± 4.4</td>
<td>67.6 ± 4.5</td>
</tr>
<tr>
<td>Pooled human saliva (73 ± 2)</td>
<td>66.2 ± 3.2</td>
<td>32.8 ± 2.8</td>
<td>27.4 ± 4.5</td>
</tr>
<tr>
<td>Bench-dried (NA)</td>
<td>No viable cells</td>
<td>No viable cells</td>
<td>No viable cells</td>
</tr>
</tbody>
</table>

* Mean values are represented in percentage of viable cells.
† mOsm/kg