

## Milk as an interim storage medium for avulsed teeth

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### Abstract

*In this study, milk was evaluated as a potential storage medium for avulsed teeth. In various experiments, storage in milk was compared to storage in saliva, in water, and to air drying. The results demonstrate that milk is significantly superior to saliva, water, and air in preserving periodontal ligament fibroblast viability. Based on these results, it is recommended that when an avulsed tooth cannot be reimplanted immediately, it should be placed in milk and brought to the dentist for reimplantation.*

Several techniques have been suggested for interim preservation of avulsed teeth until reimplantation can be accomplished. Immediate replacement of the avulsed tooth into the socket is the soundest biological approach,<sup>1</sup> but replacement rarely is accomplished at the time of the traumatic injury. Because nonprofessionals hesitate to implement this technique, several procedures for interim storage have been suggested.

Some investigators have recommended placing the avulsed tooth into tap water while others suggest placing it in the buccal vestibule where it is bathed by saliva.<sup>2</sup> Recently, Blomlof and coworkers recommended commercial bovine milk as an interim storage medium.<sup>3,4</sup> Advantages of milk as a preservative fluid are that it is nearly isotonic and is relatively free of bacterial contamination. Other investigators have demonstrated the presence of metabolically active and viable cells that are secreted with human colostrum.<sup>5</sup> These data indicated that milk may be an effective temporary storage medium for periodontal ligament (PDL) fibroblasts associated with the roots of avulsed teeth.

The purpose of this study is to compare the efficacy of commercial bovine milk to other suggested storage media in the maintenance of PDL fibroblast viability, human skin fibroblast viability, and the proliferative capacity of human skin fibroblasts.

### Methods and Materials

#### Viability of PDL Cells

Extracted premolars and third molars, were used to evaluate PDL cell viability. They were free of caries and advanced periodontal disease. Immediately after extraction, the teeth were placed in Hank's buffered saline<sup>a</sup> (HBS) at a temperature of 4°C. All remnants of blood, gingiva, and osseous tissue were removed under sterile conditions with HBS washes, tissue forceps, and a surgical blade. Teeth were kept immersed in HBS during this procedure. These procedures were accomplished within 30 minutes of tooth extraction.

Media tested for their ability to preserve the viability of PDL fibroblasts were: (1) HBS as a positive control, (2) pooled whole human saliva, (3) tap water, and (4) commercial pasteurized whole bovine milk. Treatment of the extracted teeth consisted of immersion in one of the experimental media for 1 hour at 20°C. Additionally, one group of teeth was allowed to air-dry 1 hour at 20°C. A total of five teeth were used in each group.

After this incubation period, fibroblasts associated with the PDL were removed enzymatically by collagenase (0.15% collagenase for 2 hours at 37°C).<sup>b</sup> (The teeth were placed in culture tubes containing 5 ml of collagenase during the treatment.) After the collagenase treatment the culture tubes were vortexed and the teeth removed. The remaining cell suspensions of fibroblasts then were centrifuged (800 g for 10 minutes) and washed three times in HBS. Next, the fibroblasts were suspended in 0.5 ml of HBS and diluted 1:10 in 0.05% trypan blue diluted with 0.9 N NaCl. The percentage of viable and nonviable cells were determined using hemocytometer counts. Fibroblasts were considered nonviable if they could not exclude trypan blue or if they exhibited ballooning degeneration. Significant differences in each group were determined using Student's t-distribution.

<sup>a</sup> Gibco, Grand Island, N.Y.

<sup>b</sup> Collagenase #C-0130 Sigma, St. Louis, Mo.

### Viability of Human Skin Fibroblasts

Because of difficulties in PDL fibroblast quantitation and viability determination, human foreskin fibroblasts were used in the following two groups of experiments. Human foreskin fibroblasts have been shown to be analogous to PDL fibroblasts and easily can be quantitated. The cells were obtained and maintained in culture as previously described.<sup>6</sup> All fibroblasts were used between the second and fifth serial passage and were proliferating actively (noncontact inhibited). A total of  $5 \times 10^5$  human fibroblasts in 2.5 ml of tissue culture medium (RPMI 1640 supplemented with 10% fetal calf serum, 50 units of L-glutamine, and 10 ug/ml of gentamycin) were placed in 5 ml culture tubes. The cells were pelleted (800 g, 10 minutes) and the supernatant aspirated.

The various preservation media were layered gently over cell pellets that were approximately 0.2 mm in depth. The preservation media then were incubated with the cell pellets for 1 hour at 20°C. After this incubation the test media carefully were removed and the cell pellet resuspended in HBS. Cell viability and recovery rates were determined by the trypan blue dye exclusion method previously described. Significant differences between experimental groups were determined using Student's *t*-distribution.

### Proliferative Ability of Human Foreskin Fibroblast

The proliferative ability of experimentally treated human foreskin fibroblasts was determined by a tritiated thymidine (<sup>3</sup>H-TdR) incorporation technique.<sup>6</sup> Briefly, fibroblasts in these assays were prepared and treated with experimental media as in the assay for fibroblast viability. In these experiments both actively proliferating and sessile (contact inhibited) fibroblasts were used. The saliva, milk, and tap water were sterilized by ultrafiltration through a 0.45 m filter apparatus.<sup>c</sup>

After incubations with the experimental media the fibroblasts were resuspended in 5 ml of tissue culture media, and 0.2 ml aliquots were placed in cells of a microtiter tissue culture plate.<sup>d</sup> Each well was immediately pulsed with 0.2 μCi of <sup>3</sup>H-TdR<sup>e</sup> and the fibroblasts cultured 24 hours at 37°C in a 5% CO<sub>2</sub> atmosphere. After the culture period, the fibroblasts were collected and washed with an automatic cell harvester (MASH). Fibroblast proliferative capacity was determined by <sup>3</sup>H-TdR incorporation into DNA as measured by liquid scintillation counting. Statistical differences between experimental groups were determined using Student's *t*-distribution.

## Results

### PDL Cell Viability

The viability of PDL fibroblasts, determined by trypan

<sup>c</sup> Gelman, Ann Arbor, Mich.

<sup>d</sup> Falcon, Cockneyville, Md.

<sup>e</sup> ICN Chemical and Radioisotope, Irvine, Calif.

**Table 1.** Viability of PDL Fibroblasts After Interim Storage Procedures<sup>1</sup>

Medium <sup>2</sup>	HBS	Milk	H <sub>2</sub> O	Saliva	Air
% Viable Viability <sup>5</sup>	88.2 ± 2.6	32.5 ± 2.4 <sup>3</sup>	15.0 ± 7.9 <sup>3,4</sup>	16.7 ± 4.3 <sup>3,4</sup>	17.0 ± 2.9 <sup>3,4</sup>
	1.0	.37	.17	.189	.19

<sup>1</sup> Viability by trypan blue dye exclusion. <sup>2</sup> N=5 for each group; value represent  $\bar{x} \pm$  S.E.M. <sup>3</sup> *p* < .0001 when compared to HBS (Student's *t*-distribution). <sup>4</sup> *p* < .001 when compared to milk (Student's *t*-distribution). <sup>5</sup> Viability Index = exp. medium viability ÷ positive control (HBS) viability.

blue dye exclusion after a 1-hour incubation in the various preservation media, is shown in Table 1. The order of PDL fibroblast preservation was as follows: HBS, milk, saliva, air drying, and water. Teeth stored in saliva and tap water yielded a large number of fibroblasts that exhibited ballooning degeneration. The total number of fibroblasts recovered from the incubations with tap water, saliva, and air drying was less than the milk and HBS groups.

### Viability and Recovery of Fibroblasts After Treatment With Experimental Media

The viability and recovery of human foreskin fibroblasts were evaluated after treatment with various experimental media. A total of  $5 \times 10^5$  fibroblasts with an initial viability of 89.2% were used in each replicate group. After a 1-hour incubation with each of the experimental media, viability with respect to the original cell number ( $5 \times 10^5$ ) was calculated using the trypan blue exclusion method (Table 2). Milk demonstrated significant superiority in maintaining fibroblast viability when compared to saliva, tap water, or air drying. The

**Table 2.** Viability of Human Fibroblasts After One-Hour Treatment with Preservation Media<sup>1</sup>

Medium <sup>2</sup>	HBS	Milk <sup>3</sup>	Saliva <sup>3,4</sup>	H <sub>2</sub> O <sup>3,4</sup>	Air <sup>3,4</sup>
Percent viable ± S.D.	80.6 ± 3.7	59.3 ± 5.9	33.9 ± 4.8	5.6 ± 0.8	7.8 ± 2.5

<sup>1</sup> Viability determined by trypan blue dye exclusion (initial viability 89.2%). <sup>2</sup> N=5 for each group. <sup>3</sup> *p* < .001 when compared to HBS (Student's *t*-distribution). <sup>4</sup> *p* < .001 when compared to milk (Student's *t*-distribution).

**Table 3.** Recovery of Human Fibroblasts After One-Hour Treatment with Preservation Media<sup>1</sup>

Medium <sup>2</sup>	HBS	Milk <sup>3</sup>	Saliva <sup>3,4</sup>	H <sub>2</sub> O <sup>3,4</sup>	Air <sup>3,4</sup>
Percent recovery ± S.D.	82.5 ± 4.7	70.9 ± 6.6	51.3 ± 7.80	57.6 ± 11.2	39.3 ± 4.2
Recovery <sup>5</sup> index	1	0.86	0.62	0.69	0.48

<sup>1</sup> Recovery in relation to total original fibroblasts. <sup>2</sup> N=5 for each group. <sup>3</sup> *p* < .01 when compared to HBS (Student's *t*-distribution). <sup>4</sup> *p* < .01 when compared to milk (Student's *t*-distribution). <sup>5</sup> Recovery Index = exp. group ÷ positive control (HBS).

total number of fibroblasts available for cell counting after the incubation also was greater with the milk group (Table 3). This enhanced recovery of fibroblasts confirmed the observation in the first group of experiments that indicated possible fibroblast lysis in the saliva, tap water, and air-dried groups.

#### Proliferation of Fibroblasts After Treatment With Experimental Media

The ability of human foreskin fibroblasts to proliferate after a 1-hour incubation in the experimental media was evaluated by a <sup>3</sup>H-thymidine incorporation assay. Results of these proliferation assays are shown in Table 4. Experiment 1 was performed with contact-inhibited fibroblasts while experiment 2 used rapidly proliferating fibroblasts. In both experiments the proliferative ability of fibroblasts was maintained best by the positive control (HBS) and milk.

#### Discussion

Traumatic injuries to young permanent teeth frequently result in avulsion.<sup>1</sup> A number of investigators have demonstrated that these teeth may be reimplanted successfully.<sup>7,8</sup> They also have shown conclusively that reimplantation success depends on the time elapsed between avulsion and reimplantation. It has been suggested that the predominant reason for reimplantation failure in traumatically avulsed teeth is the lack of a viable PDL.<sup>2,9,10</sup> Death of the PDL fibroblasts and denaturation of collagen associated with the PDL is likely to stimulate inflammatory resorption after tooth reimplantation.<sup>1,11</sup> A number of factors, such as desiccation or immersion in hypotonic fluids, lead to decreased viability of the PDL and, thus, enhance the possibility of inflammatory resorption. Another possible cause of reimplantation failure is bacterial contamination of the PDL. Bacterial products may have direct toxicity to PDL and also may accentuate the postreimplantation inflammatory response.<sup>11</sup>

These results agree with the findings of Blomlof and coworkers,<sup>3,4</sup> and indicate that milk is a superior interim storage medium for avulsed teeth. They extend previous findings by showing that milk is an effective agent in maintaining the proliferative ability of fibroblasts such as those associated with the PDL. This is an important finding since proliferation of the PDL may be essential for successful reimplantation.

While the experiments gave some insight into the efficacy of various media in maintaining PDL fibroblast viability, objective evaluation of this data was difficult. Since variable numbers of PDL fibroblasts were lost due to lysis during incubation in the test media, these fibroblasts were not available for viability counting. In addition, accurate viability counts could not be obtained by a simple count of the cells present. Because it is impossible to quantitate accurately the total number of PDL fibroblasts associated with an extracted tooth, the level

**Table 4.** The Ability of Human Fibroblasts to Proliferate After Treatment with Test Media<sup>1</sup>

Test Medium <sup>2</sup>	Experiment 1		Experiment 2	
	CPMS $\bar{x}$ + S.D.	% Control	CPMS $\bar{x}$ + S.D.	% Control
HBS	1172 ± 130		3155 ± 321	
Milk	891 ± 215 <sup>3</sup>	76.0	2797 ± 410	88.6
Saliva	204 ± 17 <sup>3,4</sup>	17.4	830 ± 88 <sup>3,4</sup>	26.3
H <sub>2</sub> O	61 ± 11 <sup>3,4</sup>	5.2	59 ± 6 <sup>3,4</sup>	1.9

<sup>1</sup>Proliferation determined by <sup>3</sup>H-thymidine incorporation assay. <sup>2</sup>N=6 for each group, 1-hour incubation. <sup>3</sup>p < .01 when compared to HBS control. <sup>4</sup>p < .01 when compared to milk group.

of fibroblast lysis that was occurring during experimental treatment could not be determined. Another variable that was difficult to quantitate was the effect of collagenase on fibroblast viability. The 2-hour collagenase treatment may have caused lysis of fibroblasts that were sublethally damaged by the experimental treatments.

Because of these shortcomings, additional experiments investigating the effects of the various test media on known numbers of human fibroblasts were undertaken. Human foreskin fibroblasts were used in these studies because of metabolic and morphologic similarities to PDL cells.<sup>12</sup> Fibroblast cell pellets approximately 0.2 mm in depth were used to simulate the thickness of the human PDL. The efficacy of milk in maintaining skin fibroblast viability in these experiments was greater than in the experiments with extracted human teeth. A factor that may be involved in this discrepancy is that the collagenase treatment which may have affected fibroblast viability adversely in the first group of experiments was not used in the subsequent experiments. The recovery index of fibroblasts from the experimental media indicated that cell lysis was taking place. The recovery rate was significantly lower in the saliva, water, and air-dried groups than for the milk group. This suggests that fibroblast viability counts from extracted teeth are not accurate, since many of the original fibroblasts already may have lysed at the time of counting.

The ability of fibroblasts to proliferate after incubation with an experimental medium should be a significant parameter in evaluating the long-term viability of these cells. The results of the human foreskin fibroblast experiments indicate that fibroblasts incubated with milk were more capable of proliferating than the other experimental incubation groups (Table 4). In fibroblasts that were proliferating rapidly (experiment 2, Table 4), the ability of these cells to proliferate after treatment with milk was not statistically different from their ability to proliferate after treatment with the positive control (HBS).

Viability of the PDL fibroblasts is the most important factor in successful tooth reimplantation. These cells rapidly deteriorate and are destroyed if they are exposed to air, water, or saliva for even 1 hour. Because of this, the length of time the avulsed tooth is exposed to one

of these media has been a determining factor in successful reimplantation. These studies show that milk significantly improves the viability of PDL fibroblasts after 1 hour when compared to other recommended storage media such as saliva or water. This is significant in that storage of the avulsed tooth in milk may extend the time which the tooth can be out of the socket and still be reimplanted successfully.

Immediate replacement of the tooth into the avulsion site is still the soundest approach, but because many non-professionals hesitate to do this, placement of the tooth in milk is a reasonable alternative.

## Conclusions

The results of this study indicate that:

1. Milk is a significantly better preservative of PDL fibroblast viability and proliferative ability than is saliva, water, or air-drying, but not as good as HBS.
2. Milk meets the three criteria for an interim storage medium for avulsed teeth: (1) it is able to preserve the viability of PDL cells; (2) it is relatively free from bacteria; and (3) it commonly is available while HBS is not.
3. Storage of an avulsed tooth in milk may extend the time the tooth can be out of the socket and still be reimplanted successfully.

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## Quotable Quote

Classroom teachers have known and reported for years that reading disability can run in families. When children without intellectual or emotional handicaps, nevertheless, have severe problems with reading and writing, educators have observed, such "dyslexia" tends to show up frequently in siblings and parents.

Beginning with this anecdotal evidence, scientists in recent years have been searching for clues to the genetic transmission of dyslexia, and recently a team of psychogeneticists reported preliminary evidence that reading disability indeed is passed from generation to generation. The suggested mode of inheritance, moreover, may lend support to an emerging theory linking learning disability, handedness, migraine headaches, and immune dysfunction to the hormones controlling early neurological development . . .

From: Herbert, W. Scientists find hereditary form of dyslexia. *Science News*, Vol. 123, No. 12, March 19, 1983.