

Original Article

## Success of replantation of avulsed teeth using human colostrum as an interim storage medium

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Received 6 October 2022; revised 5 December 2022; accepted 8 February 2023; Available online 19 February 2023



### المخلص

**أهداف البحث:** يعرف قلع الأسنان بأنه إزاحة كاملة للأسنان من التجويف السنخي وأفضل علاج هو إعادة زرع السن. يؤثر حليب الأم على صحة الجسم ونموه وتطوره المرتبط بوجود مكونات غذائية دقيقة وكبيرة. صممت الدراسة لتسجيل تأثير اللبأ البشري كوسيط تخزين على نجاح إعادة زرع السن.

**طريقة البحث:** تم إشراك ثلاثين من ذكور الجرذان البالغة في قلع القاطع الأيسر العلوي ووفقاً لوسط تخزين الأسنان المقتطعة لإعادة الزرع، تم تقسيم الجرذان إلى 3 مجموعات. مجموعة محلول هانك ومجموعة مياه الصنبور ومجموعة اللبأ. تم إجراء اختبار الجدوى باستخدام 3- (4،5-ثنائي ميثيل ثيازول-2-يل) -2،5-ثنائي فينيل -2 لفحص المواد والتقييم النسيجي، وقدر التحليل النسيجي في فترة 45 يوماً بعد الجراحة لتحديد نخر اللب، والتطعيم اللثوي، والنسبة المئوية للمنطقة التي تمت إعادة امتصاصها وربط الرباط اللثوي.

**النتائج:** أظهر وسط اللبأ نسبة مئوية أعلى من صلاحية الخلية متبوعاً بمحلول هانك مع قيمة فرق كبيرة. أظهرت النتائج النسيجية للسن المخلوع المعاد زرعه والذي تم غرسه في ماء الصنبور كوسيط تخزين ارتشاف جذر خارجي وداخلي واضح. نخر اللب وتلطيخ الرباط اللثوي مع وجود اختلافات كبيرة في النيم مقارنة بمحلول هانك ومجموعات اللبأ. بينما تظهر مجموعة اللبأ رابطاً جديداً معاد ربطه جيداً مع اللب الطبيعي ولا يوجد دليل على ارتشاف الجذر.

**الاستنتاجات:** يتم تقليل فقدان الأسنان إلى الحد الأدنى باستخدام اللبأ البشري كوسيط تخزين في إعادة زرع الأسنان المخلوعة بعد فترة ساعة واحدة مقارنة بمحلول هانك والماء.

**الكلمات المفتاحية:** اللبأ؛ وسط التخزين؛ زراعة الأسنان؛ ارتشاف الجذر؛ الرباط اللثوي؛ محلول هانك

### Abstract

**Objectives:** Tooth avulsion is defined as total tooth displacement from the alveolar socket; the best treatment is replantation of the tooth. Human milk influences body health, growth, and development related to the presence of micro and macro nutrient components. This study assessed the effects of human colostrum as a storage medium on the success of tooth replantation.

**Methods:** The upper left incisor of 30 adult male Wistar rats was extracted, and the rats were divided into three groups according to the storage medium of the extracted tooth for replantation: Hank's balanced salt solution (HBSS), tap water, and colostrum groups. The MTT cell viability assay was conducted, and histological evaluation and histomorphometric analyses were performed on postoperative day 45 for identification of pulp necrosis, periodontal hyalinization, percentage of resorbed area, and the periodontal ligament attachment.

**Results:** The colostrum medium showed a higher percentage of cell viability followed by HBSS with statistical significance. Histological findings of the replanted avulsed tooth that soaked in tap water as storage medium showed obvious external and internal root resorption.

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Peer review under responsibility of Taibah University.



Pulp necrosis and periodontal ligament hyalinization and with a significant differences values in comparison to HBSS and colostrum groups ( $p > 0.05$ ), whereas the colostrum group showed new well-reattached periodontal ligament with normal pulp and no evidence of root resorption.

**Conclusion:** Tooth loss is minimized by using human colostrum as storage medium in replantation of an avulsed tooth after a 1 h period compared to HBSS and water.

**Keywords:** Colostrum; Hank's balanced salt solution; Periodontal ligament; Root resorption; Storage medium; Tooth replantation

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## Introduction

Complete displacement of a tooth from its socket is referred to as avulsion; the treatment of choice for this case is tooth replantation to restore oral function and esthetic.<sup>1,2</sup> The prognosis of the healing process of replantation and its success depends on many factors including the health of the subject, root status, type of storage medium, and how long the avulsed tooth remains.<sup>3,4</sup>

Restoring the tooth attachment with alveolar bone and gingival tissue is the goal of treating of an avulsed tooth, which occurs with formation of new bone and new cementum, reestablishment of the periodontal connection, and restoration of blood to the pulp and the surrounding tissues.<sup>5,6</sup> Avoiding complications of replantation such as inflammation or resorption may prevent loss of the tooth.<sup>7,8</sup>

Hank's balanced salt solution (HBSS) is used as storage medium containing essential nutrients, and has the ability to maintain the viability of periodontal ligament (PDL) cells.<sup>9,10</sup> Moreover, it acts as a mitogenic agent for periodontal fibroblasts, which affects the success of replantation of the avulsed tooth.<sup>11,12</sup> Milk is also recommended as a transport medium for avulsed teeth by its ability to maintain PDL cell viability.<sup>13</sup>

Colostrum is milk that is secreted by the mammary gland the first 4–5 days after a baby is born. It is rich in carotenoids, proteins, calcium, sodium, immunoglobulins (Igs) (especially IgA), and vitamins (mainly vitamin A). Zinc is also present in the colostrum at concentrations reportedly 17 times higher than in maternal circulation, whereas lactose and fat levels are low.<sup>14,15</sup> Many studies have used HBSS and milk as storage media,<sup>16,17</sup> but no study on the use of colostrum has been reported.

The purpose of the study was to show the effects of using colostrum as storage medium for 1 h on the regeneration and reconstruction of dental tissues after replantation of an avulsed tooth compared to HBSS and tap water.

## Materials and Methods

### *In vitro study (viability test) by the MTT assay*

The NIH 3T3 murine fibroblast cell line (No. 93061524; Sigma, St. Louis, MO, USA) was cultured in Dulbecco's modified Eagle medium. Cells were seeded in a 96-well microplate at a density of  $1 \times 10^4$  cells per well in 100  $\mu$ L and cultured at 37 °C and 5% for 24 h. Then cells were treated with one sample from each of the three storage media (HBSS, tap water, and colostrum) for another 24 h followed by the MTT cell viability assay (Sigma). After 72 h, colorimetric estimation of cell proliferation and viability was carried out at 570 nm as described by Wang et al.<sup>18</sup> The percentage of viable cells was calculated and analyzed statistically by one-way analysis of variance (ANOVA) as follows:

$$\text{Percentage viability} = \frac{\text{absorbance of test samples} - \text{absorbance of the blank}}{\text{absorbance of control well} - \text{absorbance of the blank}} \times 100$$

### *In vivo study*

#### *Animal models*

Thirty adult male Wistar rats, weighing 0.35–0.40 kg and aged 4–6 months, were utilized in this study. All experiments were performed according to ethical approval from the ethics committee of Al-Mustaqbal University College (License No: 069229; Hillah, Iraq) and under the National Council's guidance. The animals were kept at a temperature of 22 °C and constant humidity and divided into the following three groups according to the storage medium used for the avulsed tooth: HBSS, tap water, and colostrum groups.

#### *Collection of colostrum*

The colostrum (30 mL) was collected from a 24-year-old female volunteer after 1 day of normal labor. Before collection, she took a bath with warm water to stimulate flow of the colostrum and the nipple area was cleaned. Small drops of colostrum that began to appear were kept in a sterile container at 4 °C for no longer than 24 h.

#### *Extraction and replantation*

Ketamine–xylazine anesthesia (0.1 mL/10 g body weight, Rompun®; Germany) was administered intraperitoneally to the rats. The incisors were washed with an antiseptic, and extraction was carried out with a scalpel blade (#15) and with application of little force to extract the tooth without any trauma. Treatment of the extracted tooth consisted of immersion in one of the storage medium (HBSS, tap water, and colostrum) for 1 h at 20 °C. A total of 10 teeth were used for each group. After 1 h, the extracted teeth were replanted into their respective socket and splinted using phosphoric acid (etching) for 1 min, followed by washing and drying,

bonding, and application of a flowable composite resin. A systemic antibiotic (penicillin–dihydrostreptomycin, 1 mL per 25 kg intramuscular injection) was given for 3 days postoperatively after replantation to prevent wound infection. Animals were sacrificed with an overdose of diethyl ether on postoperative day (POD) 45, after which the anterior portion of the maxilla was dissected.

#### Histological investigation

The specimens were decalcified in 10% formic acid, washed, dehydrated, and embedded in a wax block. Then serial sections were selected to involve the middle and apical third of the root. The slides were stained with hematoxylin and eosin (H&E).

Using Image Lab Software 2.3 to analyze the PDL, the cementum and percentage of resorbed area were analyzed using the following formula: total resorbed area  $\times$  100 and divided by the total tooth area.<sup>19</sup> The data were analyzed statistically using the fissure test and Student's *t*-test.

#### Results

##### MTT results

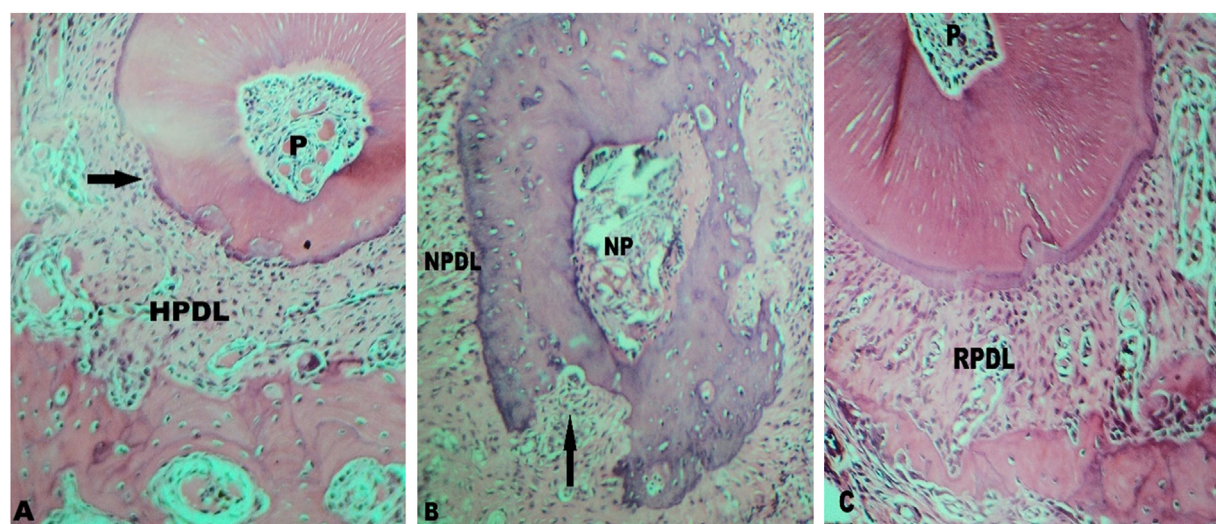
The MTT assay was conducted after 72 h to determine cell viability (Table 1). Cells incubated in colostrum media had a higher percentage of cell viability ( $89.657 \pm 6.611$ ) followed

**Table 1: Results of the MTT assay.**

Storage medium	N/CV	Mean%	Std. deviation	Std. error	95% Confidence interval		Minimum	Maximum
					Lower bound	Upper bound		
HBSS	6	78.645	5.564	2.664	73.716	83.545	71.34	84.22
Water	6	63.112	11.976	5.433	49.546	69.765	44.66	70.54
Colostrum	6	89.657	6.611	4.123	82.733	94.600	80.65	96.46
Test of homogeneity of variances								
MTT								
Levene statistic		df1	df2		Sig.			
3.263		2	14		0.042			

**Table 2: ANOVA test for the MTT assay.**

	Sum of squares	df	Mean square	F	Sig.
Between groups	1887.121	2	966.040	11.722	0.001
Within groups	1380.720	15	84.021		



**Figure 1:** Microscopic views of the replanted avulsed tooth treated with three experimental storage materials. (A) HBSS group showed hyalinization of the PDL (HPDL), resorbed cementum (arrow), and pulp with hyaline. H&E  $\times$ 4, (B) Tap water group showed necrotic PDL (NPDL), necrotic pulp (NP), and root resorption including cementum and dentine (arrow). H&E  $\times$ 4, (C) Colostrum group showed new well-reattached PDL (RPDL) with normal pulp, and no evidence of root resorption was detected. H&E  $\times$ 4.

**Table 3: Frequency of histological parameters and F-test at POD 45.**

Parameters	No. of specimens/group	HBSS	Tap water	Colostrum	F-test	Sig.
Pulp necrosis	10	1	8	0	5.511	0.001*
PDL hyalinization	10	2	8	1	6.457	0.001
Root resorption	10	1	7	0	5.555	0.001*
Reattached PDL	10	6	1	9	8422	0.001*

\*Statistically significant at  $p \leq 0.05$ .

**Table 4: Percentage of root resorption of study groups at POD 45.**

Storage medium	Percentage of resorption	T-test	p value
HBSS	5.21 ± 1.22	7.432	<0.01*
Tap water	18.56 ± 2.23		
Colostrum	2.15 ± 1.33		

\*Statistically significant at  $p \leq 0.05$ .

by HBSS ( $78.645 \pm 5.564$ ) with statistical significance ( $p > 0.001$ ) as determined by ANOVA (Table 2).

#### *Histological and histomorphometric findings*

Histological results for the HBSS group on POD 45 revealed resorbed cementum in some areas at the apex of the root with repaired deposit of new cementum in other places. Pulp and periodontal ligament showed hyalinization in their tissue. No evidence for bone resorption was recorded, and only one specimen showed root resorption (Figure 1A). Microscopical results of the tap water group on POD 45 showed external and internal root resorption including the cementum and extending to the dentin. Pulp and PDL necrosis was observed (Figure 1B). On POD 45, the pulp, dentin, and cementum of the colostrum group showed normal histological features, while the PDL showed good rearrangement and attachment to the surrounding bone (Figure 1C). The frequency of histological parameters was non-significant among the groups regarding PDL hyalinization, and significant differences in pulp necrosis and root resorption were observed in the tap water compared to HBSS and colostrum groups. Periodontal reattachment was assessed in all study groups and a significant difference was observed for the colostrum compared to the HBSS and tap water groups (Table 3). Significant differences in root resorption were observed among groups ( $p < 0.01$ ; Table 4).

#### **Discussion**

Interim storage of avulsed teeth until replantation was evaluated with different techniques, because of the difficulties in replacing the tooth in its socket at the time of injury.<sup>20</sup> Thus, this study used several procedures for interim storage and estimated the changes in histological events that occurred during storage of the avulsed teeth and replantation until the recording of the success of replantation.

Many studies have used the cell viability test to evaluate the effects of the examined storage medium on the growth and viability of fibroblast cells.<sup>21,22</sup> Fibroblasts are derived from the germinal layer, which has the same origin of

osteogenic cells, at the same time representing the formative cell for PDL, which is an important tissue in the success of replantation process. The MTT assay is based on the reduction of MTT dye into insoluble formazan, after which the number of viable cells is calculated.<sup>23</sup> The results showed that storage in the colostrum medium resulted in a higher percentage of viable cells compared to storage in HBSS and tap water medium. Histological features including pulp necrosis, periodontal hyalinization, percentage of resorbed area, and PDL attachment were assessed for all replanted avulsed teeth after storage in different media (HBSS, tap water, and colostrum) for a 1 h period.

At POD 45, the HBSS group had resorbed cementum in some areas with deposits of new cementum and no evidence of bone and root resorption. These results were in agreement with Souza et al.,<sup>25</sup> who reported that milk and HBSS are more effective in maintaining cell viability and proliferation capacity than any other storage media. HBSS has long been used for cell maintenance and tissue preservation because of its ideal pH and osmolality that help in preserving cell vitality; therefore, it has been widely employed as a positive control in studies related to dental avulsion.<sup>24,25</sup>

On the other hand, some specimens showed hyalinization in the tissues of pulp and PDL, which may have been related to the solution used at 20 °C as many studies prefer to use solution at 37 °C to obtain full efficacy as preservative media. Moreover, it has been recommended that the avulsed tooth be placed in HBSS for no more than 30 min,<sup>26,27</sup> whereas our study extended the storage period to 1 h.<sup>26,27</sup>

The tap water group had pulp and periodontal necrosis and extensive root resorption; these findings may be related to the poor characteristic properties of water including having a non-ideal pH and osmolality with a chance of bacterial contamination. Moreover, tap water is a hypotonic solution that has no ability to maintain cell morphology and may result in visible destruction and rapid cell lysis and death, as the present results showed failure to keep the avulsed tooth alive. These results are in accordance with De Brier et al., who found that tap water was the least desirable storage medium.<sup>10,27</sup>



The colostrum group showed success in replantation, as the histological findings showed normal pulp tissue and reattachment of the PDL with no root resorption observed for the examined specimens. These results contribute to the facts related to human milk as a whole including its physiological properties as being sterile, free from bacterial contamination, and an isotonic fluid in which cells can survive.<sup>28</sup> Moreover, colostrum is rich in carotenoids, proteins, specific electrolytes calcium, sodium, IgA, vitamin A, and zinc. All of these nutrients are important for cell survival, proliferation, and the reestablishment of new tissue.<sup>29,30</sup>

The results of this study suggest the use of colostrum as a storage medium because it significantly preserves the viability of dental cells compared to water and HBSS. In addition, because it is sterile and free of bacteria, it helps with the storage and transport of avulsed teeth.<sup>31</sup> Moreover, it is easy to collect and is free of cost compared to HBSS.

## Conclusion

This study demonstrated the efficacy of colostrum as an excellent storage medium in preserving avulsed teeth for a long period of time. It is a significantly better preservative and transport media than HBSS and tap water.

## Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Conflict of interest

The authors have no conflict of interest to declare.

## Ethical approval

All experiments were performed according to ethical approval from the ethics committee of Al-Mustaqbal University College (License No: 069229) and under the National Council's guide for the care of laboratory animals.

## Authors' contributions

AYH contributed to the design of this work. LKK analyzed the data. AHSh interpreted the data. MFJ discussed the results. MMA checked the whole manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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**How to cite this article:** Al-hijazi AY, Al-khafaji LK, Shaalan AH, Jabaz MF. Success of replantation of avulsed teeth using human colostrum as an interim storage medium. *J Taibah Univ Med Sc* 2023;18(6):1189–1194.