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Cling film as storage medium for avulsed teeth

An in vitro pilot study

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SUMMARY

The long-term prognosis of avulsed teeth primarily depends on the behavior at the scene of the accident. Lay people are not able to perform an immediate replantation. Therefore, particular significance belongs to the cell-physiologic storage of avulsed teeth. The aim of this pilot study was to evaluate whether cling film facilitates the survival of periodontal ligament cells in vitro. For this purpose, healthy human third molars were used. They were cut into root slices, which were stored in one of five test media: SOS Zahnbox®, UHT milk (4 °C), sterile isotonic saline solution, tap water, and cling film. Following storage periods of 2 hours, 6 hours, and 24 hours in the respective medium, slices were cultivated at 37 °C and 5% CO_2. After 2 days, 7 days, and 14 days in culture, surviving periodontal ligament cells of each slice were assessed quantitatively. Apart from tap water, all investigated media promoted cell survival. At the time of 2 hours, storage in cling film facilitated the highest cell growth compared to all other media. At the time of 6 hours, teeth stored in cling film showed cell growth comparable to that observed in the SOS Zahnbox®. The results of this pilot study indicate that cling film possibly could be used as an alternative transport medium for a storage period of up to 6 hours.

Introduction

Tooth injuries caused by accidents are frequent and affect more than half of all adolescents up to the 16th year of life (Borssen & Holm 1997; Diaz et al. 2010). In as many as 7.4% of the cases, injuries involve avulsions (Gassner et al. 1999). Owing to their exposed position, maxillary incisors are most often affected (Borssen & Holm 1997; Brunner et al. 2009; Ivancic et al. 2009; Fariniuk et al. 2010; Orlando et al. 2010), in over 78% of the cases, the maxillary central incisors (Cunha et al. 2001; Nik-Hussein 2001).

The long-term prognosis of avulsed teeth primarily depends on the behavior at the scene of the accident. The goal of the tooth rescue chain is the preservation of reversibly damaged tissue on the root surface (Pohl 2008). The occurrence of substitution resorption associated with ankylosis is mainly due to non-physiologic storage media as well as to the duration of storage (Werder et al. 2011). Among other things, immediate replantation of the tooth is generally recommended (Andersson et al. 2012), although over 90% of interviewed lay people do not feel capable to perform themselves a replantation at the scene of the accident (Jorge et al. 2009). This emphasizes the practical significance of cell-physiologic storage media for the transport from the place of accident to the dentist.

The optimal storage medium is determined both by its physiologic pH value and its physiologic osmolarity (Marino et al. 2000). In addition, the storage medium should exhibit anti-inflammatory and anti-microbial properties, it should contain no or only a minimal microbial burden, and it should be readily available at the place of accident and efficient (Blomlöf 1981a; Ramos & Miranda 2007).

Tooth rescue boxes developed particularly for the storage of avulsed teeth (SOS Zahnbox®, Miradent Co., Hager & Werken GmbH Co., Duisburg, Germany; Dentosafe®, Medice GmbH Co., Iserlohn, Germany) contain nutrients, amino acids, and vitamins as well as an integrated pH-buffer. In this way, they maintain the survival and proliferation capacity of periodontal ligament cells (PDL cells) for at least 24 hours at room temperature (Pohl & Kirschner 1994; Pohl et al. 1999). Studies confirm the efficacy of the tooth rescue boxes as physiologic nutritional medium in terms of the survival and proliferative rate of PDL cells (Pohl et al. 1999; Pohl et al. 2005). Teeth stored physiologically attained the highest degree of periodontal healing in vivo (Werder et al. 2011). Since tooth rescue boxes are not available always and everywhere, alternative storage media are described in the literature.

Milk was investigated extensively and obtained acceptance as storage medium (Blomlöf & Otteskog 1980; Blomlöf et al. 1980a, 1980b; Blomlöf 1981a, 1981b; Courts et al. 1983; Huang et al. 1996; Marino et al. 2000; Sigalas et al. 2004; Thomas et al. 2008; Souza et al. 2010a, 2010b; Moazami et al. 2012; Souza et al. 2012). The almost neutral pH value and a physiologic osmolarity, a bacterial burden kept down by the pasteurization procedure as well as the content in growth factors and nutrients turn cold and ultra high temperature (UHT) milk into a cost-effective and readily accessible storage medium (Blomlöf 1981a; Belford et al. 1995; Gauthier et al. 2006; Khademi et al. 2008).

By virtue of its isotonicity, sterile saline solution is recommended as a further alternative, however it lacks the necessary nutrients for the metabolism of the PDL cells (Blomlöf 1981a). Although some in vitro studies suggest the efficacy of the saline solution as storage medium, this disagrees with the experience in clinical daily routine (Patel et al. 1994; Pileggi et al. 2002; Subramaniam et al. 2015).

The suitability of water as storage medium was investigated repeatedly. As a consequence of its hypotonicity, it entails rapid bursting and thus the death of the PDL cells (Khademi et al. 2008). Storage of avulsed teeth in water for more than 20 minutes generally led to root resorption (Andreasen et al. 1995). In several studies dealing with water as storage medium, a comparatively low or even the lowest survival rate of PDL cells was observed (Courts et al. 1983; Marino et al. 2000; Pearson et al. 2003; Casaroto et al. 2010; Souza et al. 2010a, 2010b, 2010c; Moazami et al. 2012; Souza et al. 2012; Silva et al. 2013; Ghasempour et al. 2015).

Similar to that in water, dry storage is regarded as an unphysiological storage method. Frequent occurrence of substitution resorption correlated with the duration of unphysiological extraoral storage of the avulsed tooth (Werder et al. 2011).

The present study evaluates cling film as a possible transport medium. It is efficient, comparatively easy of access similar to milk, and owing to its capability to retain the body fluid on the root surface, it could have a positive effect on the survival of the PDL cells. The aim of this in vitro study was to evaluate the efficacy of cling film in terms of cellular survival within a period of up to 24 hours.

Materials and Methods

The study was carried out in accordance with the ethical principles of the Helsinki declaration. Thus, patients in an informed consent prior to the intervention agreed to provide their teeth for the purpose of the study. Thirty-five caries-free, partially or fully retained third molars were used. For their surgical removal flap elevation, but no osteotomy was required. Immediately following extraction (duration of dry storage: 0 minutes), seven teeth, each, were randomly assigned to one of the following test groups: SOS Zahnbox®, UHT milk (4 °C), sterile isotonic saline solution, water (tap water), and cling film. Throughout the subsequent storage period of as many as 24 hours, milk constantly was kept cool, while the other media were used at room temperature. Two hours after the surgical removal, teeth were pretreated for the following cultivation. To this end, they were grasped at the crown with maxillary incisor forceps (Ustomed Co., Tuttlingen, Germany) and inserted in a holder. The root tip was resected using a diamond-coated disc (Komet Dental Co., Brasseler Bros. GmbH & Co. KG, Lemgo, Germany), and the pulp was removed using a Retropost® drill (Komet Dental Co., Brasseler Bros. GmbH & Co. KG, Lemgo, Germany) and external cooling with sterile saline solution (B. Braun Melsungen Co., Melsungen, Germany). Subsequently six root slices, as evenly thick as possible, were cut off with the diamond-coated disc. In order not to alter the microbial burden of the medium, all six of the slices as well as the tooth crown and the root tip were returned into the medium. Two root slices, each, were randomly withdrawn from the medium after 2 hours, 6 hours, and 24 hours. This corresponded to the length of stay (LOS) in the medium. Immediately following withdrawal, each slice was rinsed for a minute, each, in 500 µl PBS (phosphate buffered saline) and 500 µl DMEM (Dulbecco's modified Eagle's medium) and then fixed in a 24-well culture plate (Falcon®, Becton Dickinson Co., Franklin Lakes, USA). As culture medium 300 µl DMEM was used, and the duration of cultivation was 14 days at 37 °C and 5% CO_2. Every 48 hours, the culture medium was replaced, and the root slices were checked for contamination using a light microscope (Leica DM IL LED, Leica Co., Wetzlar, Germany).

The pH value of the storage medium in the SOS Zahnbox® as well as of UHT milk, sterile isotonic saline solution, and water was determined prior to the start of the investigation and at the withdrawal times of 2 hours, 6 hours, and 24 hours.

The thickness of the slices was measured at the end of the examination using a digital caliper (Henry Schein Inc., Melville NY, USA).

After a proliferation period (PP) of 2 days, 7 days, and 14 days, cell growth was analyzed. For this purpose, the root slices were subdivided in four virtual quadrants. At four-fold microscopic magnification, the cell growth of each slice was classified into one of the following predefined rates: Rate 0 (no cell growth), rate I (at the maximum 10 cells in a quadrant), rate II (more than 10 cells in a quadrant), and rate III (more than 10 cells in several quadrants). If different rates of cell growth were found in a root slice, the highest rate was taken into account for the subsequent evaluations. Since two intraindividual root slices existed for each withdrawal time (2 hours, 6 hours, and 24 hours), the arithmetic mean of the rates was used for the statistical analysis.

Considering that cell growth over the entire proliferation period (2 days, 7 days, and 14 days) in general was uniform or progressive, contaminated samples were included in the findings

Tab. I Descriptive summary of all possible influencing factors in the various test groups

	SOS Zahnbox®	UHT milk	Saline solution	Water	Cling film	Significance test
Age (years)	24.1	29.4	26.3	28.1	24.3	$F[4,35] = 0.48$, $p = 0.75$
Percentage of females	42.9	85.7	57.1	28.6	85.7	$F[4,35] = 2.09$, $p = 0.11$
Percentage of maxillary teeth	71.4	85.7	71.4	57.1	57.1	$x^2(4, N = 35) = 1.86$, $p = 0.76$
Slice thickness (mm)						
LOS[1] 2 hours	0.69	0.75	0.69	0.72	0.55	$F[4,35] = 1.19$, $p = 0.34$
LOS[1] 6 hours	0.70	0.76	0.70	0.64	0.55	$F[4,35] = 1.15$, $p = 0.24$
LOS[1] 24 hours	0.65	0.60	0.72	0.61	0.66	$F[4,35] = 0.49$, $p = 0.74$

[1] Length of stay

Tab. II Average pH value of the examined media over the length of stay (LOS) of 24 hours

	SOS Zahnbox®	UHT milk	Saline solution	Water
LOS 0 hours	7.00	6.50	5.00	7.00
LOS 2 hours	7.00	6.50	5.07	6.71
LOS 6 hours	7.00	6.50	5.43	6.93
LOS 24 hours	7.00	6.50	5.64	7.07

Tab. III Number of contaminated root slices over the length of stay (LOS) of 24 hours

	SOS Zahnbox®	UHT milk	Saline solution	Water	Cling film
Overall	3	5	2	7	1
LOS 2 hours	1	1	0	2	0
LOS 6 hours	1	1	1	2	0
LOS 24 hours	1	3	1	3	1

Tab. IV Probability of cell growth (%) over the length of stay (LOS) of 24 hours

	LOS 2 hours	LOS 6 hours	LOS 24 hours
SOS Zahnbox®	43	71	43
UHT milk	57	86	57
Saline solution	100	71	57
Water	29	0	14
Cling film	86	86	43

by means of the Last-observation-carried-forward method (LOCF).

In addition to the rate of cell growth, the probability of cell growth was calculated. This is the probability with which a cell in the respective medium will grow over the proliferation period of 14 days. The basis for this was a binomial classification of cell growth: Rate 0 was defined as no growth, and rates I to III were regarded as growth. This allowed deriving the percentage of root slices with growth as a function of the storage medium and the time of withdrawal (2 hours, 6 hours, and 24 hours).

The statistical analysis was made using SPSS (version 22.0). For interval and nominal scaled data, analysis of variance (ANOVA) and the chi-square test was applied, respectively. The level of significance was set at 5% ($p \leq 0.05$).

Results

The average age of the 35 patients examined was 26.5 years (SD = 8.52 years). The sample comprised 21 females (mean age = 23.29 years; 18–36 years; SD = 3.68 years) and 14 males (mean age = 31.21 years; 19–63 years, SD = 11.11 years). No significant differences between the test groups became evident in terms of age, gender, and the site of removal of the third molar (maxilla vs. mandible; Tab. I). Likewise hardly any differences were found with respect to the thickness of the root slices, which varied by 0.21 mm at the maximum.

In the case of the SOS Zahnbox® the pH value over 24 hours remained constant at 7.00 (Tab. II). The same was true for milk, although the pH was somewhat lower, amounting to 6.50. In the case of the saline solution it varied only insignificantly in a slightly acidic range ($p = 0.096$), and in the case of water it fluctuated around pH 7.00 ($p = 0.669$).

Contaminations occurred most frequently in the medium water (Tab. III).

In the test group cling film the probability of cell growth at the withdrawal time of 2 hours was 86% and thus very high (Tab. IV). Up to the time of 6 hours it remained constant, then decreased to about half (43%) at 24 hours. An even higher probability of cell growth became evident only in the case of the sterile saline solution (100% at the time of 2 hours). The probability of cell growth in the medium of the SOS Zahnbox® attained the highest value of 71% at the time of 6 hours,

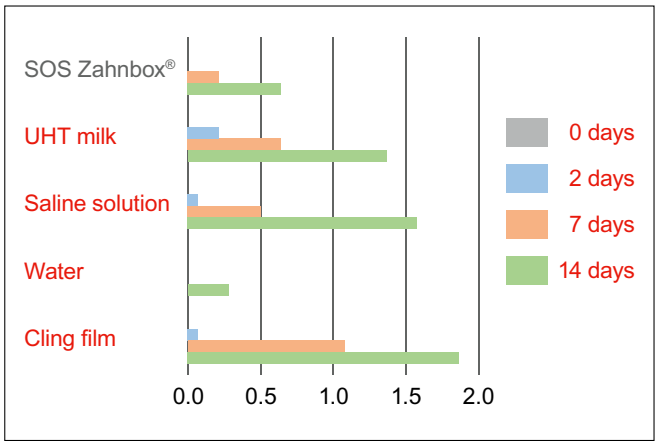

Fig. 1 Bar graph illustrating the rates of cell growth over proliferation periods of 0 days, 2 days, 7 days, and 14 days following a 2-hour length of stay in the SOS Zahnbox® as well as in UHT milk, saline solution, water, and cling film

although this was still lower than in the case of the cling film. Milk also revealed the highest value of 86% at the time of 6 hours, whereas in the medium water the probability of cell growth was lowest at all points in time.

In all test groups, cell growth at the withdrawal time of 2 hours increased in parallel to the duration of proliferation (2 days, 7 days, and 14 days; $p < 0.001$; Fig. 1). Compared to all of the other media cling film revealed the highest rate of cell growth ($p = 0.033$). At the time of 6 hours, the SOS Zahnbox® was the sole medium to show the highest cell growth. However statistically significant differences between the test groups did not become apparent. The same applied to the withdrawal time of 24 hours, except in the case of water after a proliferation period of 7 days. Only in the case of the cling film, storage for 24 hours resulted in inferior cell growth (PP 7 days: $p = 0.017$; PP 14 days: $p = 0.005$).

Finally, potential differences between the test groups SOS Zahnbox® and cling film were examined. Cell growth of all teeth which were stored for 2 hours in cling film across all periods of proliferation (2 days, 7 days, and 14 days) was markedly higher than that observed in the case of the SOS Zahnbox® ($p = 0.041$).

Discussion

Numerous studies have dealt with the evaluation of storage media in relation to the survival of PDL cells. The goal of the present in vitro pilot study was to ascertain, in what way cling film could be used as transport medium for avulsed teeth.

In vitro studies on animal teeth demonstrated deleterious effects exerted by dry storage of up to 90 minutes on the survival of PDL cells as well as on the frequency of ensuing substitution resorption (Dos Santos et al. 2009; Mori et al. 2010; Barbizam et al. 2015). After dry storage of 120 minutes, no living cells could be established (Soder et al. 1977). The occurrence of substitution resorption correlated with the duration of dry storage (Chappuis & von Arx 2005). Whereas in previous studies dry storage repeatedly resulted in cell death (Donaldson & Kinirons 2001; Casaroto et al. 2010; Werder et al. 2011), cling film used in the present investigation at all withdrawal times (2 hours, 6 hours, and 24 hours) revealed cell growth comparable to the other test groups. At the time of 2 hours, storage in cling film entailed even higher cell growth than in the SOS Zahnbox®.

A possible explanation for the good results obtained with the cling film is its ability to keep the tooth moist in an environment rich in nutrients. Whereas dry storage on air leads to desiccation of the cells and storage in a handkerchief results in absorption of fluid and hence of nutrients, cling film is capable of retaining the body fluid on the root surface. This allows PDL cells to subsist themselves over a certain time. The substantial decrease of cell growth at the withdrawal time of 24 hours could equally suggest that the nutrients still present at the beginning were depleted by the cells.

UHT milk cooled down to 4 °C at all withdrawal times (2 hours, 6 hours, and 24 hours) revealed cell growth comparable to that observed with the SOS Zahnbox®. These results are in agreement with those of other in vitro studies (Ashkenazi et al. 1999; Silva et al. 2013). Milk principally is suited only for a short duration of storage (Courts et al. 1983; Huang et al. 1996; Moazami et al. 2012), but it is worth mentioning that lower temperatures promote the viability of cells (Blomlöf & Otteskog 1980; Blomlöf 1981a; Sigalas et al. 2004). Cooled milk demonstrated greater survival of PDL cells compared to the tooth rescue box (Marino et al. 2000; Souza et al. 2010b) and principally better results than milk at room temperature (Blomlöf & Otteskog 1980; Blomlöf 1981a; Ashkenazi et al. 1999). Low temperatures have the advantage that they entail a reduction of the cellular metabolism and of bacterial growth (Barile 1994; Ashkenazi et al. 1999). The factor temperature thus could account for the comparable findings obtained in this study with milk and the SOS Zahnbox®.

Tooth rescue boxes can maintain the viability and proliferative capacity of PDL cells for at least 24 hours at room temperature (Pohl et al. 1999). The SOS Zahnbox® used in the present study revealed the highest degree of cell growth at the withdrawal time of 6 hours. This comparatively short preservation of viability could be accounted for by the duration of the extraction procedure: For the operative removal, force is applied to the tooth for a prolonged length of time, whereas in the case of the avulsion, already 1.5 milliseconds are sufficient for the sudden rupture of the PDL (Miura & Maeda 2008). Therefore, the extraction procedure can damage more PDL cells than the avulsion event.

Sterile saline solution at all withdrawal times (2 hours, 6 hours, and 24 hours) yielded results comparable to the other test groups. Similar findings were obtained in other in vitro studies (Patel et al. 1994; Pileggi et al. 2002; Subramaniam et al. 2015). Saline solution as the sole medium revealed a cell growth probability of 100% at the time of 2 hours. Teeth stored in cling film likewise exhibited a high growth probability of 86%. Here cells from about four out of five root slices proliferated. Since a declining trend as a function of increasing lengths of stay theoretically would be anticipated with all storage media examined, the present results could also indicate a possible methodical fault.

These findings suggest that the probability of cell growth could constitute an additional practice-relevant criterion for the selection of the optimal storage medium. Subsequent studies should not only evaluate the survival of the PDL cells, but also its probability.

Moreover it has to be mentioned that the present in vitro study is not quite realistic insofar as teeth never were partially or completely dried out. This can indeed happen in reality until a storage medium is available. Possibly differences would emerge in this respect, because the fluids exhibit a higher rehydration

capacity and hence more likely facilitate the survival of perhaps battered, but still vital cells.

In summary it can be concluded that cling film, by retaining the thin fluid film on the root surface, can create a physiologic environment necessary for cell survival. Within the first 6 hours, this is comparable to that in a tooth rescue box. Water however is not suited as storage medium for avulsed teeth.

The low number of samples in our pilot study limits the possible interpretation of the actual findings. Additional and large-scale in vitro as well as in vivo studies are necessary to confirm the good preliminary results regarding cling film as a possible transport medium.

Résumé

Le prognostic à long terme des dents avulsées dépend principalement du comportement sur la scène de l'accident. Les profanes ne sont pas capables de réaliser une réimplantation immédiate. Par conséquent, le stockage physiologique cellulaire pour les dents avulsées relève d'une importance particulière. Le but de cette étude pilote était de savoir si le film alimentaire soutient la survie des cellules PDL in vitro.

A cet effet, des dents de sagesse humaines saines ont été utilisées. Celles-ci ont été coupées en tranches de racine et stockées dans l'un des cinq milieux examinés: SOS Zahnbox®, le lait UHT (4 °C), la solution saline aseptisée isotonique, l'eau du robinet et le film alimentaire. Après une durée de 2 h, 6 h et 24 h, les tranches ont été cultivées pour 14 d à 37 °C et 5% CO_2. Après 2 d, 7 d et 14 d, la survie cellulaire de chaque tranche a été analysée quantitativement.

Sauf l'eau du robinet, tous les milieux examinés soutiennent la survie cellulaire. Après 2 h, le stockage dans le film alimentaire a permis une croissance cellulaire plus forte, comparé aux autres milieux. Après 6 h, les dents stockées dans le film alimentaire ont montrée une croissance cellulaire similaire à celle dans SOS Zahnbox®.

Les résultats de cette étude pilote suggèrent que le film alimentaire pourrait être utilisé comme un milieu du transport alternatif pour une période de stockage de 6 h.

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