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# The effects of three modified Hank's balanced salt solutions on root resorption of late replanted teeth: A pilot study

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#### ABSTRACT

*Purpose:* The purpose of this study was to evaluate the effects of replanted rats' teeth that had been soaked in one of three modified Hank's balanced salt solutions (HBSSs) before replantation and after extended extra-oral dry time.

*Materials and methods:* Maxillary right incisors were extracted from 55 Wistar rats and kept dry for 30 or 60 min (n = 5 each). Afterwards, the pulp was extirpated and both the papilla and enamel organ were removed with a scalpel. Each group of teeth was soaked in one of three modified HBSSs or HBSS alone. After 30 min of immersion in solutions, the root canals were dried and filled with calcium hydroxide paste, and the teeth were replanted. After 8 weeks, animals were euthanized; then, specimens were processed as 5  $\mu$ m-thick serial sections for histological examination and morphometric assessments.

*Results:* The percentages of root resorption for the groups were found to be in the following order: HBSS3 (the bFGF group) > the HBSS only group > HBSS2 (the GSH group) > no soaking (the positive control group) > HBSS1 (the ALN group) for 30 min and the positive control group > the HBSS3 only group > HBSS2 > HBSS3 > HBSS1 for 60 min. The lowest incidence of resorption was observed in immediately replanted teeth (negative control).

Conclusions: The findings of this study suggest that soaking for 30 min in HBSS containing 1 mM alendronate can significantly inhibit root resorption for avulsed teeth that have been dried for 60 min. © 2018 European Association for Cranio-Maxillo-Facial Surgery. Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

Avulsions are severe dental injuries involving complete traumatic displacement of the injured tooth from its socket, leading to pulp tissue injury in the apical region, periodontal injury, and accompanying injury of the surrounding tissues, such as the supporting bone and cement (Andreasen et al., 2007). The reported incidence of dental avulsion ranges from 1% to 16% of all traumatic injuries to permanent dentition (Andreasen et al., 2007; Carvalho et al., 2012; Mori et al., 2013). Replantation is the treatment of choice, but it cannot always be performed immediately (Flores et al., 2007). The extra-alveolar dry period and storage medium in

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which the tooth is kept prior to replantation remain critical factors affecting the healing of replanted teeth (Carvalho et al., 2012; Barbizam et al., 2015).

To ensure healing, the avulsed tooth should be replanted as soon as possible (Gulinelli et al., 2008). A drying time of less than 5 min is ideal, while 15–20 min is considered acceptable for periodontal healing to be expected (Andreasen, 1981). A drying time of 60 min or more is considered too long for the survival of the root periodontal ligament cells, and resorption over most of the root surface is expected (Trope, 2011). Often patients arrive at the emergency clinic with avulsed teeth that have dried for more than 20 min and less than 60 min. In these cases, the root surface consists of some cells that have the potential to regenerate, and some that will act as inflammatory stimulators (Trope, 2011). Soaking the tooth in an enriching medium before replantation washes dead cells from the root surface and can potentially revitalize the surviving cells, minimizing the inflammatory response and reducing root resorption (Pettiette et al., 1997).

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Hank's balanced salt solution (HBSS) is essentially a pH-balanced salt solution containing ingredients, such as calcium, glucose and magnesium ions, that can sustain and reconstitute the depleted cellular components of the periodontal ligament cells (Ashkenazi et al., 2001). HBSS is relatively inexpensive and has a shelf life of 2 years at room temperature. Additionally, it has shown a superior ability to maintain the viability of periodontal ligament fibers for extended periods (Ashkenazi et al., 2001; Moazami et al., 2012; Chen et al., 2015). However, a previous study that tried soaking roots in various media before replantation reported a benefit only with Viaspan (Belzer UW-CSS, DuPont Pharmaceuticals, Wilmington, DE, USA); soaking in HBSS did not favor healing of replanted teeth that had dried for extended periods (Pettiette et al., 1997).

Viaspan is a medium used for organ transplant storage (Sangappa et al., 2014), and it has proven to be an extremely effective cold storage medium for organs before replantation (Todo et al., 1989). As a storage medium for teeth, Viaspan has also proven to be comparable to HBSS in preserving the periodontal ligament (PDL) (Ashkenazi et al., 2001). However, Viaspan is expensive and not readily available to the public. By identifying the active components of Viaspan, the aim is to develop a modified HBSS-based medium containing those components. Buttke et al. (Buttke and Trope, 2003) suggested that reduced glutathione (GSH) in Viaspan might function as an antioxidant agent by facilitating the decomposition of hydrogen peroxide  $(H_2O_2)$  to water, thus preserving PDL vitality by limiting oxidative damage.

Alendronate (ALN) is a third-generation bisphosphonate with demonstrated osteoclast inhibitory activity that can slow the resorptive process after traumatic avulsion without inhibiting cementogenesis at the treatment dose (Levin et al., 2001). ALN is currently used in treating osteoclast-mediated diseases such as osteoporosis, Paget's disease, and osteolytic malignancies of the bone (Mori et al., 2007).

Basic fibroblast growth factor (bFGF, FGF-2) is a member of the heparin-binding growth factor family that can induce angiogenesis, chemotaxis, and proliferation of undifferentiated ectodermal cells in PDL (Takayama et al., 1997). Studies have shown that topical application of bFGF promotes the formation of a new periodontal ligament and reduces the occurrence of ankylosis and root resorption after tooth replantation (Seshima et al., 2010; Shiratani et al., 2012; Tuna et al., 2015). Takayama et al. (1997) suggested that bFGF may play a role in wound healing by inducing the growth of immature PDL cells, which accelerates periodontal regeneration.

The purpose of this study was to examine the effects on replanted rats' teeth that had been soaked in one of three modified HBSSs before replantation and after they had been dried for 30 or 60 min. Thus, a comparison of the three different aforementioned substances was carried out to determine which supplement could make HBSS a more effective storage medium for avulsed teeth.

#### 2. Materials and methods

The protocols were approved by the Animal Ethics Committee of Tongji University (Shanghai, China). All animal experiments were performed under specific pathogen-free conditions in accordance with institutional guidelines. Fifty-five male Wistar rats (Rattus norvegicus, albinus), weighing 250–300 g, were used. The animals were kept in cages and identified according to the treatment group and experimental period. They were fed grained solid food and water ad libitum, except for the 12 pre- and postoperative hours.

### 2.1. Preparation of three modified HBSSs

A solution of 1 mM ALN (Sangon Biotech Co., Ltd, Shanghai, China) in HBSS (Hangzhou Genom Biological Pharmaceutical Technology Co., Ltd, Hangzhou, China) was defined as HBSS1. A solution of 5 mM GSH (reduced L-glutathione, Sigma—Aldrich Trading Co., Ltd, Shanghai, China) in HBSS was defined as HBSS2. A solution of 10 ng/ml bFGF (Human FGF-basic, Pepro Tech Inc., New Jersey, America) in HBSS was defined as HBSS3.

#### 2.2. Surgical intervention

For surgical interventions, the animals were anesthetized with 2% sodium pentobarbital (Merck KGaA, Darmstadt, Germany) by intraperitoneal injection, at a dose of 0.3 ml per 100 g of animal weight. Anesthesia was applied with a disposable insulin syringe, which was followed by antisepsis of the anterior maxillary region with 2% chlorhexidine solution. Afterwards, the maxillary right incisor of all animals was extracted as atraumatically as possible, simulating tooth avulsion.

After tooth extraction, each animal was identified for later replantation of teeth in their respective sockets. The teeth were then randomly divided into the groups listed in Table 1. Two groups of 25 teeth were kept dry and attached by the crown to a sheet of pink wax for 30 min and 60 min. The teeth were stored at room temperature. Both the dental papilla and enamel organ of each tooth were removed with a #15 scalpel blade (Shanghai Yuheng Electronic Technology Co., Ltd, Shanghai, China), and the pulp tissue was extirpated via a retrograde method using a slightly curved, barbed broach (MANI Inc., Tochigi, Japan). This removed the pulp tissue from the root apex. Root canals were irrigated with saline (Shandong Kangning Pharmaceutical Co., Ltd, Shandong, China), which was followed by aspiration. Each group was subdivided into five groups of five teeth each that were either soaked for 30 min in three modified HBSSs or HBSS alone, or were not soaked (positive control). After immersion, the root canals were dried with sterile paper points (Zhenjiang Jianerle dental equipment Co., Ltd, Zhenjiang, China) and filled with a calcium hydroxide paste (Longly Biomedical Co., Ltd, Wuhan, China) injected from the root apex. Five additional roots were extracted — these root canals were instrumented as quickly as possible and replanted immediately without drying or soaking, thus serving as a negative control.

The sockets were gently irrigated with saline (no other irrigation was used), and the teeth were replanted. Due to the stability of the replanted teeth, splinting of the teeth was deemed unnecessary. All animals received a single intramuscular injection of 20,000 U.I. of benzatine G penicillin (Sangon Biotech Co., Ltd, Shanghai, China). The animals received only water 12 h before and after the surgical procedures.

## 2.3. Micro-computed tomographic ( $\mu$ CT) analysis

Sixty days after replantation, the rats were euthanized with an overdose of sodium pentobarbital, which was followed by a cervical dislocation. The right and left sides of the maxilla were separated in

Table 1	
Allocation of roots to treatment groups	

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55 roots	5 roots: immediate replant	Negative control
	25 roots: 30 min dry	5 roots: replant without soaking
		(positive control)
		5 roots: soak 30 min in HBSS
		5 roots: soak 30 min in HBSS1 (ALN)
		5 roots: soak 30 min in HBSS2 (GSH)
		5 roots: soak 30 min in HBSS3 (bFGF)
	25 roots: 60 min dry	5 roots: replant without soaking
		(positive control)
		5 roots: soak 30 min in HBSS
		5 roots: soak 30 min in HBSS1 (ALN)
		5 roots: soak 30 min in HBSS2 (GSH)
		5 roots: soak 30 min in HBSS3 (bFGF)

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