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(–)-Epigallocatechin-3-gallate: a novel storage medium for avulsed teeth

Huangqin Chen*, Bin Huang*

Department of Stomatology, Xianning University, Xianning, China

Correspondence to: Huangqin Chen, Department of Stomatology, Xianning University, Xianning Road 88#, Xianning Hubei, 437100, China
Tel.: +86 0715 8151277
Fax: +86 0715 8151277
e-mail: chenhuangqin79@163.com

*These authors contributed equally to this study; co-first author.

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Abstract – The purpose of the present study was to evaluate the efficacy of (–)-epigallocatechin-3-gallate (EGCG) in maintaining the vitality of human periodontal ligament (PDL) cells when used as a storage medium for avulsed teeth prior to replantation. Thirty freshly extracted single-rooted human teeth with closed apices were randomly assigned to three experimental groups with 10 samples per group and immersed in one of the storage media: EGCG, Hank's balanced salt solution (HBSS), or milk for 2 h. The PDL cells were dissociated by an enzyme treatment with collagenase and trypsin. The cells were then labeled with 0.4% Trypan blue for the determination of viability. The result showed that EGCG group had the highest percentage of cell viability, followed by HBSS and milk group, in descending order.

Introduction

Dental avulsion is a consequence of injury that results in the complete displacement of a tooth from its alveolar socket and may affect multiple tissues, such as alveolar bone, dental pulp, and gingival mucosa. More seriously, the periodontal ligament may be ruptured and damaged by the traumatic pressure and may become infected. Such damage to the PDL can cause root resorption, tooth-bone ankylosis, or tissue necrosis, and these outcomes end in avulsed tooth replantation failure (1, 2). Teeth that are replanted immediately after avulsion usually show excellent healing and have been found to have a good prognosis (3). However, this is not always possible, and the problem of tooth preservation is very important for ensuring a successful replantation. Therefore, it is essential to develop storage solutions that can maintain the viability and function of the PDL for longer periods. Various materials have been studied for their potential to serve as a storage medium for avulsed teeth, such as HBSS, Viaspan, tap water, milk, saliva, and culture media, with differing degrees of success (4–6). However, none of the currently used media could be adopted as ideal. So, the search for a more suitable storage medium is still on.

(–)-Epigallocatechin-3-gallate (EGCG), a major polyphenol of green tea, is known to have various biological effects such as antioxidative, anticarcinogenic, antimutagenic, anti-inflammatory, antimicrobial, and antiviral activities (7). Recently, more attention has been paid to its storage capacity. It was reported that EGCG is effective in non-frozen preservation of mammalian cells and tissues like blood vessels, corneas, nerves, islet cells, myocardium, hematopoietic stem cells, etc., which might be involved in cell cycle control through the cytostatic activity (8). It was also reported that EGCG can protect cells and tissues from freeze/thaw cycle-induced oxidative stress under ultra-low-temperature preserving condition (9). Most of all, it has been demonstrated that the high viability of PDL cells of guinea pig and Beagle dog could be maintained using EGCG (10, 11). Therefore, this study was undertaken to assess the potential of EGCG, in comparison with the currently recommended materials, HBSS and milk, as a storage medium for avulsed teeth. The main objective was to assess the ability of the different media to preserve the vitality of periodontal ligament cells in simulated avulsed teeth for extended periods of time.

Materials and methods

Sample collection

Thirty healthy single-rooted teeth extracted for orthodontic reasons with no caries, restoration, periodontal disease, or hypoplasia were selected. The teeth were extracted as atraumatically as possible and washed in sterile saline solution to eliminate residual blood. Following extractions, the teeth were then randomly assigned to three experimental groups with 10 samples per group and immersed in one of the storage media: EGCG, HBSS, or milk at room temperature for 2 h. Ten teeth were allocated to the control groups. The positive control group comprised of five teeth that were neither dried nor stored in any solution, but assayed immediately for cell vitality. The negative control teeth (five samples) were dried for 8 h, with no follow-up storage solution time, and then assayed.

Preparation of EGCG and other media

The purified EGCG and HBSS were commercially procured from Sigma Chemicals Co (Santa Clara, CA,

USA). The purity of the EGCG was 98%. Working solution of EGCG was prepared by dissolving it in deionized water with the terminal concentration of 1 mg ml^{-1}. It was stored in the refrigerator at 4°C and used within 1 week. Whole pasteurized milk was used.

Cell dissociation from human periodontal ligament

After washing with phosphate buffer saline (PBS), periodontal ligament tissue was collected by scraping the root surface from the middle one-third to the apex. The tissue was disaggregated using 0.2% collagenase and 0.125% trypsin for 30 min at 37°C, and the cells were collected by centrifugation at 1000 r/min for 5 min. The pellet of the packed cells was then resuspended in T75 cell culture flasks with α-minimum essential medium containing 15% fetal calf serum (Gibco BRL Co. Ltd., Gaithersburg, MD, USA), 100 M ascorbic acid 2-phosphate (Gibco), 2 mM L-glutamine (Gibco), 100 U ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin. The flask was incubated at 37°C with an atmosphere of 95% air and 5% CO_2 for the attachment. When the dishes became subconfluent, the periodontal ligament fibroblasts were detached by applying 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) solution for 3 min at 37°C. Third- and fourth-passage periodontal ligament fibroblasts were used in this experiment to control the cell variability.

Cell viability assay

In principle, the cells with damaged membrane allow the trypan blue dye to pass through membrane into cytoplasm, whereas undamaged cells exclude dye. The number of viable cells was measured by the trypan blue dye exclusion test by assessing the loss of membrane integrity. In brief, after the cells were collected and labeled with 0.4% (w/v) of Trypan Blue solution (Sigma), enumeration of viable and non-viable cells was carried out under a light microscope with a hemocytometer at 20× magnification and analyzed.

Statistical analysis

The results are expressed as mean ± standard deviation (SD). ANOVA was employed to detect differences among the groups. $P < 0.05$ was taken to indicate significant differences.

Result

The percentage averages (±SD) of cell viability for different media are shown in Fig. 1. ANOVA analysis showed that EGCG group had the highest percentage of cell viability, followed by HBSS and milk group, in descending order ($P < 0.05$) (Table 1).

Discussion

Hammer (12) demonstrated that the length of survival of a replanted tooth is directly correlated with the amount of viable periodontal ligament. Thus, extra-alveolar period (time and storage medium) has been suggested as one among the important factors for the success of replantation of avulsed teeth (13, 14). The efficacy of EGCG as a storage medium has been explored for many tissues and cells, such as osteoblasts, fibroblasts, endothelial cells, platelets, blood vessels, nerves, articular cartilage of joints, and hematopoietic stem cells (8, 15, 16). The hypothesis that EGCG may contribute to the physiological preservation of tissue or organ, particularly rat pancreatic islets, was first proven by Hyon's observation (17). Since then, several studies have been reported showing these beneficial preservative effects. It was reported that the high cell viability of guinea pig and Beagle dog PDL cells could be maintained using EGCG (10, 11), and the ability of EGCG to control the viability of human PDL fibroblasts has also been confirmed (18).

In this study, the concentration of 1 mg ml^{-1} EGCG was used, which is lower than that in some previous studies. There were two reasons: one was that 1 mg ml^{-1} green tea polyphenol was effective on the preservation of many tissues such as peripheral nerves (19, 20) and human saphenous vein (21); the other was that the purified EGCG solution was not readily available to the common man. Only the green tea or the green tea solution could be achieved in markets which might with a low concentration of EGCG (about 1 mg ml^{-1}, 1 g tea×11%/100 ml water = 1 mg ml^{-1}, EGCG is about 11% percent of the dry weight of tea). Results showed that EGCG with lower concentration was still able to maintain PDL cells viability superior to HBSS and milk.

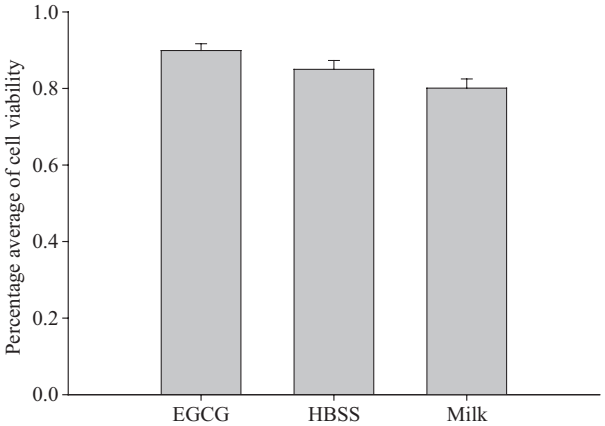

Fig. 1. Effect of EGCG on the cell viability of PDL cells. EGCG group: 0.899 ± 0.1792; HBSS group: 0.850 ± 0.2309; milk group: 0.801 ± 0.2378. EGCG, (−)-epigallocatechin-3-gallate; HBSS, Hank's balanced salt solution; PDL, periodontal ligament.

Table 1. Mean-Tukey honestly significantly different for comparison between individual groups

Groups	Asymptotic significance (two-tailed)	SE	Significance
EGCG and HBSS	0.000	0.00973	Significant
EGCG and milk	0.000	0.00973	Significant
HBSS and milk	0.000	0.00973	Significant

EGCG, (−)-epigallocatechin-3-gallate; HBSS, Hank's balanced salt solution; SE, standard error.

The preservative potential of EGCG may be related to the intrinsic characteristics of polyphenolic compounds, which readily penetrate cell membranes because of their amphipathic properties (17). These compounds combine easily with the extracellular matrix, phospholipid bilayered membranes, and any type of intracellular protein, as well as the cytoplasm and nucleus. The adsorption of polyphenolic compounds to proteins is generated early, but the desorption rate is very slow. Consequently, mammalian cells or tissues could be physiologically preserved through adsorption of the compounds to membranous proteins and collagen fibers, leading to the reduction in structural deterioration (8). Furthermore, polyphenolic compounds have been shown to trap reactive oxygen species (ROS), such as superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide, and peroxynitrite. Among tea polyphenolic compounds, EGCG is most effective in reacting with most reactive oxygen species. The chemical structures contributing to effective antioxidant activity of EGCG include the vicinal dihydroxy or trihydroxy structure, which can chelate metal ions and prevent the generation of free radicals (9). This structure also allows electron delocalization, conferring high reactivity to quench free radicals. These activities may be related to the mechanism underlying the maintenance of cell viability observed in the present study.

The promising potential of EGCG has been explored in dental parts as well as in the medical fields. It was reported that EGCG could not only extend the lifespan of dental cells and tissues and maintain them in a stable condition (10, 11), but also improve the periodontal condition because of its antibacterial, antimycotic, antioxidant, and anti-inflammatory properties. More than that, it was demonstrated that EGCG could inhibit the formation of osteoclasts (22), the production of related cytokines (23) and their inflammatory pathways (24). Preservation with EGCG can be regarded as an effective therapeutic strategy for avulsed teeth replantation, as inflammatory responses, ankylosis along with loss of cell viability in PDL cells attached to avulsed tooth because of bacterial contaminations, and oxidative stress-mediated events are well reported during extra-alveolar period. Thus, EGCG has preservative potential for use as storage medium that can promote favorable tooth replantation with lower rates of infection and less root resorption and ankylosis.

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