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Effectiveness of Green Tea Extract in Maintaining Viability of Avulsed Tooth Fibroblasts in Rats

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Research Article

Effectiveness of Green Tea Extract in Maintaining Viability of Avulsed Tooth Fibroblasts in Rats

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Abstract

Tooth avulsion represents a severe traumatic dental injury in which a tooth is forcibly expelled from its alveolar socket, potentially resulting in significant damage to the surrounding periodontal structures. The success of replantation procedures is primarily determined by the condition and survivability of periodontal ligament (PDL) fibroblast cells. To maintain cellular integrity before replantation, the use of an appropriate storage medium is essential. Green tea (*Camellia sinensis*) is a herbal plant rich in flavonoid compounds that possess antioxidant and tissue-regenerative capabilities, particularly in stimulating fibroblast proliferation. A concentration of 70% green tea extract has been identified as the most beneficial for promoting wound recovery. This investigation sought to assess how effectively green tea extract could sustain fibroblast cell viability obtained from avulsed teeth of male Wistar rats. A total of thirty-six teeth harvested from male Wistar rats were allocated into one control group and two experimental groups, each subsequently subdivided based on immersion duration of 30 and 60 minutes. PDL-derived fibroblast cells were obtained through versene-trypsin isolation, followed by staining using 0.4% trypan blue solution and cell counting via hemocytometer under light microscopy. Results demonstrated that 30-minute immersion in 70% green tea extract yielded the highest fibroblast viability (90.35%; $p = 0.004$) relative to all other groups. Although both 70% and 100% concentrations successfully maintained fibroblast viability, the 70% concentration proved more effective across both observation time points.

Keywords: Tooth avulsion; fibroblasts; green tea extract; storage medium

INTRODUCTION

Trauma refers to injuries to the body caused by changes in temperature, mechanical pressure, electricity, or chemicals that may lead to complications.¹ According to the Indonesian Basic Health Research (Riskesdas) conducted in 2018, trauma was recorded in 9.2% of the population, representing a 1% increase compared to 2013, which was only 8.2%. Approximately 44.7% of these cases occurred at home or in its surrounding environment. The prevalence of head trauma in Indonesia was recorded at 11.9%, which may pose a threat of a more

significant increase in dentoalveolar trauma.²

Specifically, dentoalveolar trauma refers to injuries that may result in luxation, avulsion, tooth fractures, alveolar bone trauma and damage to the surrounding tissues at the site of injury.³

Among the various forms of dentoalveolar trauma, tooth avulsion is characterized by the total dislodgment of a tooth out of its socket in the alveolar bone. This condition accounts for approximately 0.5% to 16% of all traumatic dental injuries involving the permanent dentition, with the highest occurrence noted among children aged 7 to 9 years.⁴ The outcome following replantation of an avulsed tooth is strongly

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influenced by the maintenance of periodontal ligament (PDL) fibroblast viability, given that these cells play a critical role in achieving successful replantation and minimizing the risk of root resorption.⁵ While immediate replantation is the ideal treatment, it is not always feasible, necessitating the use of an appropriate storage medium during the extra-alveolar period.⁴ An ideal storage medium should be capable of preserving PDL cell viability, possess antimicrobial properties, and have physiological pH (6.6-7.8) and osmolality (280-300 mOsm/L).^{6,7}

Replantation is the reinsertion of an avulsed tooth into its socket, aimed at restoring aesthetic appearance and maintaining normal tooth function to support proper alveolar ridge development.⁸ The outcome of replantation depends on preserving the preservation of periodontal ligament cells, particularly fibroblasts, that remain attached to the avulsed tooth. Periodontal ligament cells serve as a vital link between the avulsed tooth and the alveolar bone socket. It is highly recommended that replantation be performed promptly to preserve fibroblast viability and prevent root resorption. Fibroblast cell viability significantly decreases if the tooth is not replanted within 60 minutes.⁷ Consequently, the use of an appropriate storage medium is necessary to preserve and enhance the viability of periodontal ligament cells prior to replantation.⁶

Furthermore, an effective storage medium for avulsed teeth should fulfill several important criteria, including easy accessibility, antimicrobial capability, and the ability to preserve the viability of PDL cells.⁶ The American Association of Endodontists recommends HBSS (Hanks' Balanced Salt Solution) as the preferred storage medium for avulsed teeth because of its excellent biocompatibility with PDL cells. However, HBSS is not widely available in Indonesia; thus, alternative storage media that are accessible, affordable, and capable of preserving

fibroblast cell viability are needed.⁹ Several studies have reported that some easily obtainable alternatives are available on the market, one of which is green tea.⁶

Green tea is a type of tea widely used in health research. Traditionally, green tea has been used to reduce the risk of pancreatic cancer, lower the risk of diabetes, and manage uric acid levels.¹⁰ In addition, green tea exhibits a wide range of pharmacological activities, such as antioxidant, anti-inflammatory, antibacterial, and anticariogenic effects, which may contribute to improved cell survival.⁶ Green tea offers various medicinal benefits, such as promoting weight loss, reducing cholesterol, triglycerides, and glucose levels, preventing dental caries, and acting as an antimutagenic agent. Green tea contains a high level of polyphenols, which significantly impact oral health, particularly due to their antibacterial properties.¹¹ Green tea also contains flavonoids, which aid tissue regeneration during wound healing by providing anti-inflammatory effects and increasing the number of fibroblasts. Flavonoids also enhance antioxidant activity, where a higher concentration of flavonoid compounds corresponds to increased antioxidant potential.¹² Previous studies have demonstrated that green tea extract can effectively preserve PDL cell viability for up to 24 hours, maintaining more than 90% viable cells.⁷ Wound healing using green tea extract was most effective in increasing fibroblast cell count at a 70% concentration.^{13,14}

In this study, avulsed teeth from male Wistar rats were immersed for 30 and 60 minutes, as these time points are considered the golden period for tooth replantation.¹⁴ This study aimed to evaluate the effectiveness of green tea extract in maintaining the viability of fibroblast cells from avulsed teeth in male Wistar rats. The results of this research are expected to provide scientific evidence supporting the potential use of green tea extract as an alternative storage medium for avulsed

teeth, thereby preserving fibroblast cell viability and enhancing the success rate of tooth replantation.

MATERIALS AND METHOD

The present study utilized a true experimental laboratory approach with a posttest-only control group design. Such a design enables the evaluation of treatment effects in the experimental groups through comparison with the control group.¹⁵

The study subjects comprised male Wistar rats (*Rattus norvegicus*) sourced from the Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya. The inclusion criteria for the samples were male Wistar rats within the age range of 2–3 months, with body weight ranging from 150–250 grams, in good physical health, free from dental caries and periodontal disease, housed under standardized conditions, and fed the same diet throughout the maintenance period.

Sample size determination was performed by applying the Federer formula for experimental research¹⁵, as follows:

$$(t - 1) \times (r - 1) \geq 15$$

Description:

t = Total number of treatment groups

r = The number of repetitions within each group, or the sample size (n) per group

Upon calculation using the Federer formula, a total of 36 male Wistar rats were enrolled and distributed equally into 6 groups. The sampling technique applied was simple random sampling.

The research instruments used in this study were animal cages, animal scales, food and water containers for Wistar rats, maceration bottles, a macerator, a complete rotary evaporator set, plastic materials, an oven, a blender, filter paper, scalpels and scalpel blades number 11, Eppendorf tubes, needle holders, disposable syringes, mini Petri dishes, glass slides, Falcon tubes, micropipettes, centrifuges, light microscopes, hemocytometers, counters, timers, digital cameras, and handscopes.

green tea, distilled water, 96% ethanol, 70% alcohol, povidone iodine (One Med), Fetal Bovine Serum (FBS) (Gibco), 0.4% trypan blue solution, DPBS (Dulbecco's Phosphate-Buffered Saline) solution (SIGMA), Versene trypsin (Biosera), Wistar rats, rat feed (Purina 593 Convid), and drinking water for the rats provided daily.

The ethical clearance for this research was obtained from the Health Research Ethics Committee at the Institut Ilmu Kesehatan Bhakti Wiyata, Kediri, bearing approval number 006/FKG/PB.4.5/V/2022.

The tea was sorted and washed with running water. After washing, it was drained and air-dried for approximately 1 day until the water content was reduced. It was then oven-dried at 35 °C for 5 hours. The dried green tea simplicia was subsequently blended into powder form. The powdered material was placed in a maceration container and combined with 96% ethanol at a 1:10 ratio (40 g of simplicia in 400 mL solvent), and stirred until homogenized for 3 days. The maceration process was carried out over 48 hours in a sealed macerator with agitation performed once daily. the mixture was strained through filter paper to remove the solid residue. The filtrate was subsequently concentrated by means of a rotary evaporator operating at 70°C and 80 mBar until a dense extract at 100% concentration was yielded. Green tea extracts at 70% and 100% were obtained by dilution.¹⁶

All rats underwent an acclimatization phase lasting 7 days to facilitate adjustment to the laboratory setting. During this period, they were maintained in cages at room temperature with a 12-hour light and 12-hour dark cycle, with ad libitum access to food and water. Tooth extraction was performed on male Wistar rats from each group after anesthesia was induced via intraperitoneal injection of ketamine at a dosage of 40–80 mg/kg body weight. Approximately 1–1.5 hours after anesthesia administration, the left

mandibular incisor of each male Wistar rat was extracted using a scalpel and needle holder. The extraction was performed in a labial-to-lingual direction, followed by traction toward the labial side. The tooth extraction was performed atraumatically, as indicated by the absence of fractures, a residual root, and the tooth being completely removed from its socket. Following extraction, the socket was irrigated with sterile saline solution.¹⁷

The extracted rat teeth were immediately immersed following the procedure. The teeth were categorized into two immersion groups: the green tea extract immersion group and the distilled water immersion group as control. Each group consisted of 6 male Wistar rats' teeth. The experiment included 3 groups in total: a control group using distilled water, a treatment group immersed in 70% green tea extract, and an additional treatment group immersed in green tea extract at 100% concentration. In each group, 6 teeth were soaked for 30 minutes, and another 6 teeth for 60 minutes. Each tooth was retrieved from the storage solution by holding it at the crown and subsequently subjected to trypsinization to detach the cells. The procedure was performed for 30 minutes in a mini-Petri dish containing 4 mL of versene-trypsin (VT) solution, with gentle agitation using a rotomix. The VT solution was then transferred into Falcon tubes, followed by the addition of 400 µL fetal bovine serum (FBS) to neutralize trypsin activity. All tubes underwent centrifugation at 1,500 rpm for 4 minutes. Following centrifugation, the supernatant was carefully removed, and the resulting cell pellet was reconstituted in culture medium.⁹ Fibroblast cell viability was subsequently determined through the trypan blue exclusion technique using a hemocytometer to quantify the proportion of viable and non-viable cells. Cell viability assessment was further conducted via the trypan blue exclusion technique under a light microscope at 10×20 magnification. Cell counting was performed with a

hemocytometer to distinguish viable cells, which remained unstained, from non-viable cells (blue-stained) cells. Calculate of cell viability percentage use the formula:¹⁸

$$\text{Viability (\%)} = \frac{\text{viable cells}}{\text{Total cells}} \times 100\%$$

RESULTS

The data presented in Table 1 demonstrate the number of viable fibroblast cells counted using a hemocytometer, followed by the calculation of cell viability as (number of viable cells / total number of cells) × 100%. The highest number of viable fibroblast cells was observed in the group immersed in 70% green tea extract for 30 minutes, with a count of 665 cells and a cell viability percentage of 90.35%. In contrast, the control group treated with distilled water for 60 minutes exhibited the lowest viable cell count (60 cells) and a viability of 33.7%. the beginning of the word and placed as in the example.

Table 2 summarizes the results of the Shapiro–Wilk normality test. The significance values for all groups, including the control groups with 30-minute and 60-minute immersion, and the treatment groups with 70% and 100% green tea extract at both immersion durations, were greater than 0.05. These findings indicate that the data in each group were normally distributed ($p > 0.05$). Homogeneity of variance was subsequently analyzed using Levene's test, which produced a significance value of 0.02 across the 6 groups, indicating that the data did not meet the assumption of homogeneity ($p < 0.05$). The violation of the homogeneity of variance assumption in this experimental study, despite the use of randomized and controlled conditions, may be attributed to inherent biological variability in cellular response to the different storage media, particularly the bioactive green tea extracts. Additionally, minor technical variations during the multi-step cell isolation and manual counting processes could contribute to the observed data spread. As a consequence of this heteroscedasticity,

nonparametric statistical analyses, specifically the Kruskal–Wallis and Mann–Whitney U tests, were selected for further evaluation, as they do not assume equal variances and are appropriate for comparing groups under these conditions. Therefore, because the data followed a normal distribution but had unequal variances, a nonparametric test was required for further analysis. The Kruskal–Wallis test was performed to evaluate whether green tea extract produced a statistically significant effect on fibroblast cell viability in avulsed teeth from male Wistar rats. The analysis yielded a significance value of 0.000, which was below the accepted significance threshold of 0.05 ($p < 0.05$). This finding confirms that immersion in green tea extract significantly affects fibroblast cell viability across the studied groups.

Analysis of the mean rank values indicates that the treatment group immersed in 70% green tea extract for 30 minutes achieved the highest mean rank (31.83), suggesting the greatest preservation of fibroblast viability under this condition. The second-highest mean rank was found in

the treatment group immersed in 70% green tea extract for 60 minutes (28.00), which also demonstrated a substantial positive effect compared to the control groups. To identify which specific group pairs differed significantly, a post hoc pairwise comparison was performed using the Mann–Whitney U test.

The results of this analysis are presented in Table 3. Comparisons between the control and treatment groups at both immersion periods demonstrated several statistically significant differences ($p < 0.05$) in several pairs, indicating differences in fibroblast viability between the groups. Conversely, non-significant p-values ($p > 0.05$) suggest no meaningful difference between some pairs.

Overall, these results demonstrate that immersion in green tea extract, particularly at 70% concentration and 30 minutes, was effective in preserving fibroblast viability in avulsed teeth. These results support the potential application of green tea extract as a natural storage medium for avulsed teeth and its possible contribution to improved outcomes in dental trauma management.

Table 1. The percentage of fibroblast viability in avulsed teeth immersed in distilled water, 70% green tea extract, and 100% green tea extract.

	Viable Fibroblast Cells	Total Cells	Cell Viability (%)
30-minute immersion			
Distilled Water	107	265	40.37%
70% Green Tea Extract	665	736	90.35%
100% Green Tea Extract	391	513	76.21%
60-minute immersion			
Distilled Water	60	178	33.70%
70% Green Tea Extract	619	713	86.81%
100% Green Tea Extract	376	532	70.67%

Table 2. Results of data analysis using the Shapiro-Wilk test, Levene's test, and Kruskal-Wallis test.

Research Group	Mean Rank	p value		
		Shapiro-Wilk	Levene's	Kruskal Wallis
Distilled Water 30 minutes	7.83	0.053	0.2	0.00
70% Green Tea Extract 30 minutes	5.17	0.45		
100% Green Tea Extract 30 minutes	31.83	0.191		
Distilled Water 60 minutes	28.00	0.535		
70% Green Tea Extract 60 minutes	21.00	0.789		
100% Green Tea Extract 60 minutes	17.17	0.883		

Table 3. Results of data analysis using the Mann-Whitney test

Research Group	Distilled Water 30 min	Distilled Water 60 min	70% Green Tea Extract 30 min	70% Green Tea Extract 60 min	100% Green Tea Extract 30 min	100% Green Tea Extract 60 min	Std. Error
Distilled Water 30 min	-	0.2	0.004	0.004	0.004	0.004	1.78324
Distilled Water 60 min	0.2	-	0.004	0.004	0.004	0.004	5.51103
70% Green Tea Extract 30 min	0.004	0.004	-	0.109	0.01	0.004	1.45161
70% Green Tea Extract 60 min	0.004	0.004	0.109	-	0.037	0.004	1.76122
100% Green Tea Extract 30 min	0.004	0.004	0.01	0.037	-	0.2	3.06792
100% Green Tea Extract 60 min	0.004	0.004	0.004	0.004	0.2	-	1.33624

DISCUSSION

An ideal storage medium must fulfill specific biological and physicochemical criteria to be considered effective for preserving avulsed teeth. The medium must be capable of maintaining PDL cell viability, supporting ongoing mitotic activity, and facilitating the replacement of damaged fibroblasts. In addition to supporting cellular vitality, an ideal medium should possess antimicrobial, antioxidant, and antibacterial properties to prevent contamination and additional tissue damage. Equally important, the medium should have a pH and osmolality comparable to those of the human body's physiological environment.⁶ To ensure that the metabolic and physiological functions of PDL fibroblasts are maintained during the extra-alveolar period, the storage medium must provide optimal osmolality,

adequate nutrient availability, and an appropriate pH range. It has been reported that the normal metabolic activity of fibroblast cells is best sustained at an isotonic osmolality of approximately 280–300 mOsm/L and within a physiological pH range of 6.6 to 7.8.⁷

Several studies have evaluated various storage media for avulsed teeth. Among these, HBSS is considered the most ideal and has been extensively tested for its effectiveness in preserving cell viability. The American Association of Endodontists (AAE) recommends HBSS (Hanks' Balanced Salt Solution) as the preferred storage medium for avulsed teeth because of its demonstrated ability to preserve the viability of PDL cell. This medium contains essential electrolytes and glucose, which support normal cellular metabolism over extended periods. HBSS has a pH of

approximately 7.2 and an osmolality of around 320 mOsm/L, which closely resembles physiological conditions.⁶

HBSS also maintains fibroblast viability at 93.3% during the first 24 hours after tooth avulsion, demonstrating its high efficacy as a storage medium. Despite these advantages, the practical use of HBSS in real-world traumatic scenarios remains limited. It is not readily available at common sites of dental trauma, such as schools, homes, camps, or sports arenas, where individuals are often engaged in physical activities, and accidents may occur. Additionally, public awareness of HBSS as an appropriate storage medium is relatively low, and its cost can be a barrier to widespread use.^{6,19}

The findings of this study demonstrate the capacity of green tea extract to sustain fibroblast cell viability in avulsed teeth obtained from male wistar rats. The ability of green tea extract to maintain fibroblast viability in male Wistar rats was demonstrated by both the number of viable fibroblast cells and the calculated percentage of cell viability obtained using the viability formula. The number of viable fibroblast cells is influenced by immersion of the teeth in green tea extract, which contains several bioactive compounds that function as antibacterial and antioxidant agents, thereby preserving fibroblast cells. These compounds include flavonoids, saponins, tannins, and proteins.¹¹

Based on the analysis of the bioactive compounds in the green tea extract used in this study, saponins had the highest concentration at 8.06%, followed by tannins at 7.61%, flavonoids at 6.58%, and proteins at 1.05%. These four bioactive constituents are presumed to contribute to maintaining the vitality of fibroblast cells. The results indicate that saponins and tannins are the predominant compounds involved in preserving fibroblast cell vitality. Saponins play a role in fibroblast formation by exerting antibacterial activity and activating key growth factors, including transforming growth factor-beta

(TGF- β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF). TGF- β and FGF stimulate fibroblast migration and proliferation. Tannins are also believed to contribute to fibroblast formation in green tea extract. Tannins function as antioxidants and can induce TGF- β expression, thereby promoting fibroblast proliferation. Additionally, tannins can induce lymphokine production, which enhances macrophage migration. Macrophages, in turn, stimulate the secretion of growth factors, thereby increasing fibroblast proliferation.^{20,21}

In addition to saponins and tannins, other compounds in green tea extract, particularly flavonoids such as epigallocatechin-3-gallate (EGCG), contribute to fibroblast viability through multiple well-documented mechanisms. EGCG, the predominant catechin in green tea, has been shown to regulate fibroblast function in a concentration-dependent manner.²² At optimal concentrations, EGCG enhances cell survival through activation of the AKT signaling pathway and stimulates proliferation by upregulating cyclin-dependent kinases CDK2 and CDK4. Furthermore, EGCG modulates the expression of fibroblast growth factors (bFGF and aFGF), which are essential for maintaining fibroblast homeostasis.²³ Green tea catechins also exert protective effects by regulating key signaling pathways associated with inflammation and cell survival, including NF- κ B, AP-1, and MAPK pathways.²⁴

An important finding of this study is that the 100% green tea extract resulted in lower fibroblast viability (76.21% at 30 minutes, 70.67% at 60 minutes) compared to the 70% extract (90.35% and 86.81%, respectively). This phenomenon, where higher concentrations produce diminished effects, is consistent with the hormesis or dual-effect model observed for many bioactive compounds. Research has demonstrated that EGCG exhibits biphasic effects on fibroblasts: at lower

concentrations, it modulates cell cycle progression and promotes survival, while at higher concentrations, it can induce apoptosis.²² This dose-dependent toxicity may be attributed to the pro-oxidant activity of EGCG at high concentrations, where it generates hydrogen peroxide (H₂O₂) and induces oxidative stress.²⁵ Additionally, the 100% extract may create a hypertonic environment with osmolality exceeding the physiological range (280-300 mOsm/L), which can compromise cell membrane integrity and metabolic function. These findings suggest that 70% green tea extract provides an optimal balance between bioactive stimulation and cytotoxicity, making it a more suitable concentration for preserving avulsed tooth fibroblasts.

Flavonoids can increase interleukin-2 (IL-2) activity and lymphocyte proliferation. The proliferation of lymphocytes influences CD4⁺ cells, thereby activating Th1 cells. Activated Th1 cells affect Specific Macrophage Activating Factor (SMAF), which encompasses multiple molecules, including interferon-gamma (IFN-γ), that activate macrophages. Activated macrophages perform phagocytosis, produce cytokines, facilitate tissue repair (through fibroblast-stimulating factors, fibronectin, and collagenase), and secrete growth hormones (growth factors). These growth factors play a crucial role in inflammation and the mitogenic processes of fibroblasts, which are essential for wound healing. Meanwhile, proteins are vital for the wound-healing process through their roles in cellular structure formation, including the formation of fibroblasts. Protein content is also necessary for fibroblasts to interact with each other, enabling them to synthesize extracellular matrix and produce collagen. The presence of protein in green tea extract, therefore, supports increased collagen production by fibroblasts.^{12,26}

While this study provides promising evidence regarding the potential use of green tea extract as a storage medium for avulsed teeth, several limitations must be

acknowledged. First, this study utilized an animal model (teeth from male Wistar rats). Although rat models are widely accepted in preliminary dental research, the anatomical and physiological differences between rat and human PDL tissues mean that the results cannot be directly generalized to human clinical practice. Further studies using human PDL fibroblasts or clinical trials are warranted.²⁷

The immersion durations tested in this study were limited to 30 and 60 minutes. These time points were selected as they represent the "golden period" for immediate tooth replantation, as recommended by the International Association of Dental Traumatology.²⁸ However, in real-world clinical scenarios, delayed replantation is common, where teeth may remain extra-orally for several hours. Therefore, the effectiveness of green tea extract at longer time intervals (e.g., 3, 6, 12, and 24 hours) remains unknown and should be investigated in future research.

This study assessed only immediate fibroblast viability using the trypan blue exclusion method. While this is a standard technique for evaluating cell membrane integrity, it does not provide information on long-term cell survival, proliferative capacity, or functional activity.²⁹ Future studies should incorporate additional assays, such as MTT or Alamar Blue, to assess metabolic activity and cell proliferation over extended periods. Furthermore, in vivo replantation studies are needed to evaluate clinically relevant outcomes, including the prevention of inflammatory root resorption and ankylosis.

This study did not include a positive control group, such as HBSS, which is considered the gold standard storage medium for avulsed teeth. The inclusion of HBSS would have enabled a direct comparison of green tea extract's efficacy with a well-established reference standard.³⁰ The absence of this comparator limits the ability to position green tea extract relative to the current standard of

care. Future comparative studies should include HBSS to provide a more comprehensive evaluation. Despite these limitations, the findings of this study offer a strong basis for further investigation of green tea extract as a natural, accessible, and cost-effective alternative storage medium for avulsed teeth, particularly in areas where HBSS is not readily available.

CONCLUSION

This study demonstrated that green tea extract at concentrations of 70% and 100% demonstrated effectiveness in maintaining fibroblast cell viability at immersion durations of 30 minutes and 60 minutes. Among the tested concentrations, the 70% green tea extract was found to be the most effective at preserving fibroblast viability compared to the 100% extract, regardless of immersion time (30 or 60 minutes).

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REFERENCES

1. Surya T, Priyanto MH. Peran Kedokteran Forensik Dalam Pengungkapan Kasus Pembunuhan Satu Keluarga Di Banda Aceh. *Jurnal Kedokteran Syiah Kuala*. 2019;19(1):45-50. <https://jurnal.usk.ac.id/JKS/article/view/18051>
2. Ministry of Health of the Republic of Indonesia. Main Results of Riskesdas 2018. Health Research and Development Agency. Indonesia: Ministry of Health; 2018. <https://repository.badankebijakan.kemkes.go.id/id/eprint/3514/1/Laporan%20Riskesdas%202018%20Nasional.pdf>
3. Arbi TA, Novita F. Knowledge Level Againts Dental Students Handling Dentoalveolar Trauma at RSGM Syiah Kuala University. *Cakradonya Dent Journal*. 2016; 8(2):98-104. <https://jurnal.usk.ac.id/CDJ/article/view/9085/7159>
4. Inayah Y, Herdiyati Y. Penanganan Avulsi Dua Gigi Permanen Pada Anak Usia 12 Tahun. *Journal of Indonesian Dental Association*. 2018; 1(1): 86-91. <https://jurnal.pdgi.or.id/index.php/jipd/article/view/322/271>
5. Abraham Y, Christy R, Gomez-Kunicki A, et al. Management of dental avulsion injuries: A survey of dental support staff in Cairns, Australia. *Dent J (Basel)*. 2021;9(1). <https://doi.org/10.3390/dj9010004>
6. Khinda VI, Brar GS, Kallar S, Khurana H. Clinical and Practical Implications of Storage Media used for Tooth Avulsion. *Int J Clin Pediatr Dent*. 2017;10(2):158-165. <https://doi.org/10.5005/jp-journals-10005-1427>
7. Hwang JY, Choi SC, Park JH, Kang SW. The use of green tea extract as a storage medium for the avulsed tooth. *J Endod*. 2011;37(7):962-967. <https://doi.org/10.1016/j.joen.2011.03.028>
8. Demir P, Guler C, Kizilci E, Keskin G. Survival of avulsed permanent incisors in children following delayed replantation. *Niger J Clin Pract*. 2020;23(5):631-637. https://doi.org/10.4103/njcp.njcp_496_19
9. Fajriyany FR, Harijadi A, Kamadjaja DB, et al. Perbandingan Efektivitas Air Kelapa Kemasan Dan Susu UHT Sebagai Media Penyimpanan Gigi Avulsi (Comparison of Effectiveness of Bottled Coconut Water and UHT Milk as Storage Media for Avulsed Tooth). *J Oral and Maxillofacial*. 2016; 5(2):20-26
10. Anggraini T. *Proses Dan Manfaat Teh*. Padang: CV. Rumahkayu

- Pustaka Utama.
- <https://opac.perpusnas.go.id/DetailOpac.aspx?id=1076734>
11. Kurnia PA, Ardhiyanto HB, Suhartini. Potensi Seduhan Teh Hijau (*Camellia sinensis*) Terhadap Peningkatan Jumlah Sel Fibroblas Soket Pasca Pencabutan Gigi pada Tikus Wistar. *e-Jurnal Pustaka Kesehatan*. 2015; 3(1):122-127.
 12. Ibnu FR, Putu WL, Suhendra L. The Content Of The Flavonoid Compound and Antioxidant Activity Of Green Tea Extract In The Treatment Temperature And Time Brewing. *Rekayasa dan Manajemen Agroindustri J*. 2018;6(3):196-202. <https://doi.org/10.24843/JRMA.2018.v06.i03.p02>
 13. Zauharoh R, Fadholah A, Sri M, Khotimah H. Uji Efektivitas Ekstrak Etanol Teh Hijau (*Camellia Sinensis*) Terhadap Penyembuhan Luka Sayat Pada Kulit Tikus. 2020;4(2):24-29. <https://doi.org/10.21111/pharmasipha.v4i2.4209>
 14. Fouad AF, Abbott P V., Tsilingaridis G, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. *Dental Traumatology*. 2020;36(4):331-342. <https://doi.org/10.1111/edt.12573>
 15. Notoatmodjo S. Metodologi Penelitian Kesehatan. Jakarta: Rineka Cipta; 2015.
 16. Yuanita T, Tedja L, Suryani D, Drismayanti I. Comparison of the Effect of Calcium Hydroxide Combination with Cocoa Pod Husk Extract and Green Tea Extract on Fibroblast and Alp Activation. *Journal of International Dental and Medical Research*. 2021;14(4):1465-1471. <https://scholar.unair.ac.id/en/publications/comparison-of-the-effect-of-calcium-hydroxide-combination-with-co/>
 17. Luthfi M, Juliastuti WS, Risky YA, Wijayanti EH, Rachmawati AE, Asyhari NPO. Expression of fibroblast cells after extraction of Wistar rat teeth after topical application of okra fruit (*Abelmoschus esculentus*) gel. *Infect Dis Rep*. 2020;12(S1):40-43. <https://doi.org/10.4081/idr.2020.8726>
 18. Kaur I B, Faktorová I D, Peña-Díaz I P, Lukeš J, Lukes J, Kaur B. Cell Counting using a haemocytometer (Neubauer cell chamber) with fixer. *FEB*. 2018;27. <https://doi.org/10.17504/protocol>
 19. Sheth PP, Lolayekar N V., Hegde AM, Shetty VA. Evaluation of periodontal ligament cell viability in honey as a storage media at different time intervals: An in vitro study. *World Journal of Dentistry*. 2020;11(4):310-315. <https://doi.org/10.5005/jp-journals-10015-1756>
 20. Nurdiana, Ulya I, Putra IPRAP. Pengaruh pemberian gel ekstrak daun melati (*Jasminum sambac* L. Ait) terhadap jumlah fibroblas kulit dalam penyembuhan luka bakar derajat II A pada tikus putih (*Rattus norvegicus*) galur wistar. *Jurnal Ilmu Keperawatan*. 2016;4(1):1-11.
 21. Jing W, Xiaolan C, Yu C, Feng Q, Haifeng Y. Pharmacological effects and mechanisms of tannic acid. *Biomedicine and Pharmacotherapy*. 2022;154:113561. <https://doi.org/10.1016/j.biopha.2022.113561>
 22. Weisburg JH, Weissman DB, Sedaghat T, Babich H. In vitro cytotoxicity of epigallocatechin gallate and tea extracts to cancerous and normal cells from the human oral cavity. *Basic & clinical pharmacology & toxicology*. 2004;95(4), 191-200. doi:

23. https://doi.org/10.1111/j.1742-7843.2004.pto_950407.x
Han DW, Lee MH, Kim, HH, Hyon, SH, & Park, JC. Epigallocatechin-3-gallate regulates cell growth, cell cycle and phosphorylated nuclear factor- κ B in human dermal fibroblasts. *Acta Pharmacologica Sinica*. 2011;32(5), 637-646. <https://doi.org/10.1038/aps.2011.17>
24. Wang L, Lee W, Cui YR, Ahn G, Jeon YJ. Protective effect of green tea catechin against urban fine dust particle-induced skin aging by regulation of NF- κ B, AP-1, and MAPKs signaling pathways. *Environ Pollut*. 2019;252:1318-1324. <https://doi.org/10.1016/j.envpol.2019.06.029>
25. Sartippour MR, Heber D, Zhang L, Beatty P, Elashoff D, Elashoff R, Go VL, Brooks MN. Inhibition of fibroblast growth factors by green tea. *Int J Oncol*. 2002;21(3):487-91. <https://doi.org/10.3892/ijo.21.3.487>
26. Zulkefli N, Che Zahari CNM, Sayuti NH, Kamarudi A, Saad N, Hamezah H, Bunawan H, Bahasum S, Mediani A, Ahmed Q, Ismail A, Sarian M. Flavonoids as Potential Wound-Healing Molecules: Emphasis on Pathways Perspective. *Int J Mol Sci*. 2023;24(5). <https://doi.org/10.3390/ijms24054607>
27. Adnan S, Lone MM, Khan FR, Hussai SM, Nagi SE. Which is the most recommended medium for the storage and transport of avulsed teeth? A systematic review. *Dental Traumatology : Official Publication of International Association for Dental Traumatology*. 2018;34(2), 59-70. <https://doi.org/10.1111/edt.12382>
28. Tewari N, Garima J, O'Connell A, Sharawat N, Rahul M, Mathur VP, Haldar P. Appropriate Terminology for the Time Elapsed From Avulsion of a Permanent Tooth to Replantation: A Scoping Review and Delphi Consensus. *Dental Traumatology : Official Publication of International Association for Dental Traumatology*. 41(1), 13-28. <https://doi.org/10.1111/edt.12993>
29. Azzoz M, Zaghloul M, Sayed M, Soliman, Mounir N, Abdel S, N, El-Dabae W, Aly S. Efficiency of MTT and Trypan Blue Assays for Detection of Viability and Recovery of Different Frozen Cell Lines. *Egyptian Journal of Veterinary Sciences*. 2024;55(6), 1649-1657. <https://doi.org/10.21608/ejvs.2024.260687.1764>
30. Najeeb S, Siddiqui F, Khurshid Z, Zohaib S, Zafar MS, & Ansari SA. Effect of bisphosphonates on root resorption after tooth replantation - a systematic review. *Dental Traumatology : Official Publication of International Association for Dental Traumatology*. 2017;33(2), 77-83. <https://doi.org/10.1111/edt.12316>