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Original Article

Effect of Polydeoxyribonucleotide on Human Periodontal Ligament Cells as a Storage Medium for Avulsed Tooth

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Abstract

Objective: This study aimed to evaluate the suitability of polydeoxyribonucleotides (PDRN) as a storage medium for avulsed teeth. **Materials and Methods:** The viability of human periodontal ligament (PDL) cells stored in Hank's balanced salt solution and PDRN solutions (concentrations, 10, 25, 50, and 100 μg/mL) and tap water was measured using the Cell Counting Kit-8 and Live/Dead assays. In addition, Nitric oxide detection and quantitative real-time polymerase chain reaction (qRT-PCR) were performed to evaluate the anti-inflammatory effect of PDRN. **Results:** The viability of PDL cells stored in a 100 μg/mL PDRN solution was significantly higher than that of cells stored in the other solutions ($p < 0.01$). Furthermore, cells stored in 100 μg/mL PDRN solution demonstrated a significantly reduced NO production ($p < 0.0001$), and cells stored in 50 and 100 μg/mL PDRN solutions expressed significantly lower levels of tumor necrosis factor α, interleukin (IL) -4, IL-6, and IL-10 ($p < 0.01$) compared to cells stored in HBSS. **Conclusion:** The PDRN solution exhibited cell-preserving and anti-inflammatory effects on the PDL cells. The findings of this study can serve as a basis for further experiments directed at the development of an effective storage medium for avulsed teeth. [J Korean Acad Pediatr Dent 2023;50(3):347-359]

Keywords

Tooth avulsion, Storage media, Polydeoxyribonucleotide

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Introduction

A tooth avulsion is the complete dislocation of a tooth from its alveolar socket due to a traumatic dental injury[1]. Immediate replantation is recommended to

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ensure better healing of the avulsed tooth and to prevent complications[2]. In a previous study, 73% of teeth immediately replanted within 5 minutes showed favorable healing of periodontal ligament (PDL) cells[3]. However, immediate replantation at the site of injury is seldom performed due to a lack of knowledge and training about the replantation procedure, complex injuries to the periodontal tissues, or life-threatening injuries[4]. If immediate replantation is impossible, it is important to store the avulsed tooth in an appropriate storage medium to maintain the viability of the PDL cells and ensure a good prognosis for the tooth.

Several studies have investigated suitable materials as storage media for avulsed teeth. The conditions for an ideal storage medium include osmolality, a pH similar to that of blood plasma, nutrients for cell metabolism, availability, and accessibility[5]. Hank's balanced salt solution (HBSS) is considered as the gold standard due to its several favorable characteristics[6,7]. In addition, pasteurized whole milk is often recommended at the scene of an accident, due to its advantages such as high availability, accessibility, and physiological compatibility[8].

In particular, various natural products are currently being investigated for their suitability as storage media due to their cost-effectiveness and easy availability[9,10]. Some studies have shown that natural products positively affect the viability of PDL cells and may be suitable for use as storage media. One study demonstrated that green tea extract maintains the viability of PDL cells better than milk[11]. Another study evaluated the suitability of 10% propolis solution as a transport medium for avulsed teeth[12]. Delphinidin, a type of anthocyanidin found in blueberries, has also been reported to preserve the viability of PDL cells and exhibit anti-inflammatory effects[13].

Polydeoxyribonucleotide (PDRN) is a registered drug with various beneficial activities, including wound healing, therapeutic angiogenesis, and anti-inflammatory and anti-ischemic effects. It has been used for therapeutic purposes in clinical medicine[14-17]. PDRN is derived through the processing of sperm DNA obtained from chum salmon or salmon trout. It is composed of a mixture of deoxyribonucleotides with molecular weights between 50 and 1500 KDa[18]. Recently, PDRN was obtained from plants, especially seaweed, and found to exhibit wound-healing effects[19,20].

PDRN has been shown to be effective in reducing the inflammation response in human cells. It exerted anti-inflammatory effects on macrophage cells pre-treated with lipopolysaccharide (LPS)[21]. Furthermore, PDRN suppressed the production of inflammatory cytokines such as interleukin (IL) -6 and tumor necrosis factor α (TNF-α) in human chondrocytes stimulated by IL-1β[22].

The efficacy of PDRN has been explored and applied in clinical practice in the field of dentistry. A study assessing the impact of PDRN during sinus elevation surgery found that PDRN stimulated early bone formation[23]. In addition, PDRN, combined with demineralized dentin matrix, induced bone formation[24]. In a retrospective clinical study, the injection of PDRN around the wound after surgical debridement in medication-related osteonecrosis of the jaw patients enhanced wound healing and reduced the recurrence of osteonecrosis[25]. To the best of our knowledge, no study has investigated the impact of PDRN on replanted teeth to date. We expected that PDRN could exert anti-inflammatory and wound-healing effects on human PDL tissues in replanted teeth. Therefore, this study aimed to evaluate the effects of PDRN on PDL cells and determine its potential and suitability as a storage medium for avulsed teeth.

Materials and Methods

1. Research ethics

This study was conducted with the approval of the Institutional Review Board of Kyung Hee University Dental Hospital (KH-DT23004).

2. Cell culture of human PDL cells

Human PDL fibroblasts (donor's age: 19 weeks gestation, passage: 1) were obtained from ScienCell Research Laboratories (Young Science Inc., Seoul, Korea). The

cells were cultured in culture media composed of Dulbecco's modified Eagle's medium (Gibco BRL, Life Technologies, Grand Island, NY, USA) with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a 5% CO_2 atmosphere. The passage 4 cells were used to perform all experiments.

3. Preparation of storage media for PDL cells

PDRN stock at a concentration of 2 mg/mL was provided by GENOSS Company Limited (Suwon, Korea). The stock solution was diluted in HBSS (Sigma-Aldrich, Seoul, Korea) at concentrations 0, 10, 25, 50, and 100 μ g/mL to prepare PDRN media. Tap water was used as a negative control.

4. Cell viability test

1) Cell Counting Kit-8 assay

The viability of human PDL cells stored in each medium was evaluated using Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan). First, PDL cells were seeded at 5×10^3 cells/well with 200 μL of culture media into a 96-well plate. After incubation for 24 hours, the culture media were removed and the following prepared solutions were treated: (a) HBSS; (b) 10 μg/mL PDRN; (c) 25 μg/mL PDRN; (d) 50 μg/mL PDRN; (e) 100 μg/mL PDRN; and (f) tap water. The cells were stored in each solution for 0, 1, 3, 6, 12, and 24 hours and exposed to room temperature and a normal atmospheric environment to imitate the extraoral condition. Then, 20 μg of CCK-8 solution was added to each plate. After incubation for 2 hours at 37°C, the optical density was measured at 450 nm (A450) using a Benchmark Plus Multiplate Spectrophotometer (Bio-Rad, Hercules, CA, USA) to determine the cell viability. The relative cell viability (%) was calculated as follows: (A450 [treated] – A450 [blank]) ÷ (A450 [control] – A450 [blank]) × 100%. The assays were performed in triplicate, and each experiment involved three samples.

2) Live/Dead assay

The viability of human PDL cells stored in each medium was evaluated by using LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Invitrogen, Paisley, UK). PDL cells were seeded at 2×10^4 cells/well with 2 mL of culture media into a 12-well plate. After incubation for 24 hours, the culture media were removed, and the prepared solutions (HBSS, 10 μg/mL PDRN, 25 μg/mL PDRN, 50 μg/mL PDRN, 100 μg/mL PDRN, and tap water) were treated for 0, 1, 3, 6, 12, and 24 hours. The dye reagent was prepared: 5 μL of 2 μM calcein AM and 20 μL of 2 mM ethidium homodimer-1 were added to 10 mL of Dulbecco's phosphate-buffered saline (Gibco BRL, Life Technologies, Grand Island, NY, USA). The resulting solution was vortexed to ensure thorough mixing. The storage media were removed from each plate, and the staining reagent was treated for 10 minutes at room temperature. A fluorescence microphotograph of live and dead PDL cells in each storage medium was obtained three times at different sites using fluorescence microscopy (IX71; Olympus, Tokyo, Japan). The number of PDL cells was counted using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). The relative cell viability (%) was calculated as follows: the number of live cells ÷ (the number of live cells + the number of dead cells) × 100. All assays were done in triplicate.

5. Nitric oxide (NO) assay

First, 2×10^5 PDL cells were seeded into a 6-well plate and incubated at 37°C in a 5% CO_2 atmosphere for 24 hours. The culture media were removed from the plates, and each well was treated with 2 mL of HBSS and 50 and 100 μg/mL PDRN for 0, 1, 3, and 6 hours at room temperature and normal atmospheric conditions. After storage each time, the cell supernatants were collected from each well, and the NO assay was conducted using the NO Plus Detection Kit (iNtRON Biotechnology, Inc., Seoul, Korea). The supernatant samples (100 μL each) were added to a 96-well plate, and a pre-reaction was induced by mixing 50 μL of N1 buffer with each sample. After incubation for 10 minutes at room temperature, 50 μL of

N2 buffer was treated to each well to conduct the final reaction. The absorbance of each sample was measured at 540 nm using Epoch (Agilent Technologies, Santa Clara, CA, USA). The nitrite concentration was determined using the standard curve of the formula in accordance with the manufacturer's instructions. All assays were performed five times, and each experiment involved two samples.

6. RNA isolation

First, 2×10^5 PDL cells were seeded into a 6-well plate and incubated at 37°C in a 5% CO_2 atmosphere for 24 hours. The culture media were removed from the plates, and wells were treated with 2 mL of HBSS and 50 and 100 μg/mL PDRN for 3 hours at room temperature and normal atmospheric conditions. RNA was extracted from three groups of PDL cells using the AccuPrep Universal RNA Extraction Kit (Bioneer, Daejeon, Korea), according to the manufacturer's instructions.

7. Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR was performed to investigate the expression levels of inflammation-related cytokines. Total RNA (5 μg) was converted into complementary DNA (cDNA) using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). The Power SYBR Green Master Mix (Thermo Fisher Scientific, Carlsbad, CA, USA) was used to assess SYBR green fluorescence using the QuantStudio 5 Real-Time PCR Ultimate Simplicity System. The cycling conditions were as follows: denaturation for 10 minutes at 95°C, followed by 40 amplification cycles of denaturation for 15 seconds at 95°C, and annealing for 1 minute at 60°C. The cDNA levels were normalized to those of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used for PCR are listed in Table 1. All experiments were performed in triplicate.

8. Statistical analysis

SPSS software (Version 21.0; IBM Corp., Armonk, NY, USA) was used to perform the statistical analysis. One-way analysis of variance was used to validate statistical significance ($p < 0.05$), followed by Tukey' honestly significant difference post hoc test was performed to determine the statistical significance of multiple comparisons. The significance level was adjusted according to the number of groups. Data are expressed as the mean ± the standard deviation value.

Results

1. Cell-preserving effect of PDRN on human PDL cells

The results of the CCK-8 assay are shown in Fig. 1 and Table 2. At 3 hours of storage, the viability of PDL cells stored in 100 μg/mL PDRN was significantly higher than that of cells stored in HBSS and tap water ($p < 0.01$, Fig. 2).

The results of the Live/Dead assay are shown in Fig.

Table 1. Primer sequences used for quantitative real-time polymerase chain reaction analysis

Gene	Forward Primer sequence (5′ - 3′)	Reverse Primer sequence (5′ - 3′)
TNF-α	GAGATCAATCGGCCCGACTA	ACAGGGCAATGATCCCAAAG
IL-4	AAACGGCTCGACAGGAACCT	TCTGGTTGGCTTCCTTCACA
IL-6	CTGCGCAGCTTTAAGGAGTT	TAGAGGTGCCCATGCTACATT
IL-10	GAGCCAACAGAAGCTTCCAT	TAGAGGGAGGTCAGGGAAAA
GAPDH	CGAGATCCCTCCAAAATCAA	CCTTCTCCATGGTGGTGAA

TNF-α: tumor necrosis factor-α; IL-4: interleukin-4; IL-6: interleukin-6; IL-10: interleukin-10; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

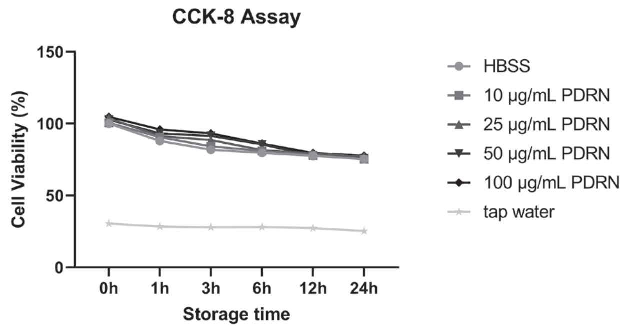

Fig. 1. Graph showing the viability (%) of PDL cells stored in each storage medium at different periods of storage time using the CCK-8 assay.
HBSS: Hank's balanced salt solution; PDRN: Polydeoxyribonucleotide.

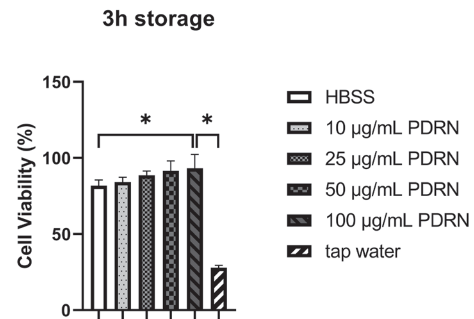

Fig. 2. Graph showing the viability (%) of PDL cells stored in each storage medium for 3 hours using the CCK-8 assay. The data are presented as the mean and standard deviation values. *$p < 0.0034$, indicating that the difference between groups was statistically significant.
HBSS: Hank's balanced salt solution; PDRN: Polydeoxyribonucleotide.

Table 2. The viability (%) of PDL cells stored in each storage medium at different periods of storage time using the CCK-8 assay

Storage time	Mean ± Standard deviation (%)					
	HBSS	10 μg/mL PDRN	25 μg/mL PDRN	50 μg/mL PDRN	100 μg/mL PDRN	Tap water
0 h	100.01 ± 9.38[a]	100.53 ± 9.38[a]	103.75 ± 13.58[a]	102.66 ± 12.39[a]	104.75 ± 14.01[a]	30.49 ± 2.33[b]
1 h	87.93 ± 6.97[a]	90.63 ± 15.57[a]	91.38 ± 10.48[a]	93.18 ± 14.05[a]	95.92 ± 9.43[a]	28.42 ± 1.56[b]
3 h	81.87 ± 3.68[a]	84.19 ± 3.13[ab]	88.51 ± 2.96[ab]	91.50 ± 6.53[ab]	93.15 ± 9.16[b]	27.91 ± 1.53[c]
6 h	79.64 ± 5.88[a]	80.93 ± 5.37[a]	81.61 ± 5.28[a]	85.61 ± 3.86[a]	86.06 ± 2.75[a]	28.04 ± 2.23[b]
12 h	77.58 ± 6.84[a]	77.93 ± 5.62[a]	79.03 ± 3.88[a]	77.58 ± 3.60[a]	79.51 ± 5.23[a]	27.26 ± 2.2[b]
24 h	75.32 ± 2.71[a]	75.36 ± 5.18[a]	77.23 ± 4.05[a]	75.84 ± 4.90[a]	77.68 ± 5.39[a]	25.30 ± 3.76[b]

Differences in lowercase letters in a row indicate statistically significant differences between storage media by Tukey' honestly significant difference post hoc test ($p < 0.0034$). HBSS: Hank's balanced salt solution; PDRN: Polydeoxyribonucleotide.

3 and Table 3. At 1 hour of storage, the viability of PDL cells stored in 100 μg/mL PDRN solution was significantly higher than that of cells stored in HBSS, 10 μg/mL PDRN solution, and tap water ($p < 0.01$, Fig. 4A). At 3 hours of storage, the viability of PDL cells stored in 100 μg/mL PDRN solution was significantly higher than that of cells stored in all other storage media ($p < 0.01$, Fig. 4B).

Fluorescence microscopic images obtained from the Live/Dead assay are shown in Fig. 5. Although the number of surviving PDL cells stored in HBSS and 10, 25, 50, and 100 μg/mL PDRN solutions decreased with the increase in storage time, the cell viability was maintained for up to 24 hours of storage. However, PDL cells stored

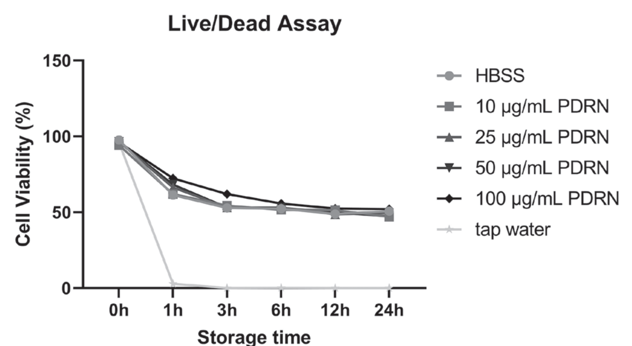

Fig. 3. Graph showing the viability (%) of PDL cells stored in each storage medium at different periods of storage time using the Live/Dead assay.
HBSS: Hank's balanced salt solution; PDRN: Polydeoxyribonucleotide.

Table 3. The viability (%) of PDL cells stored in each storage medium at different periods of storage time using the Live/Dead assay

Storage time	Mean ± Standard deviation (%)					
	HBSS	10 µg/mL PDRN	25 µg/mL PDRN	50 µg/mL PDRN	100 µg/mL PDRN	Tap water
0 h	97.58 ± 1.07[a]	94.21 ± 1.53[a]	96.42 ± 0.23[a]	96.21 ± 0.65[a]	96.06 ± 0.25[a]	95.91 ± 0.91[a]
1 h	61.35 ± 2.04[a]	61.93 ± 0.87[a]	66.26 ± 0.43[ab]	68.18 ± 2.64[ab]	72.25 ± 3.20[b]	2.86 ± 0.19[c]
3 h	52.75 ± 1.35[a]	54.28 ± 0.24[a]	53.09 ± 0.55[a]	53.46 ± 1.34[a]	61.90 ± 1.45[b]	0.00 ± 0.00[c]
6 h	52.35 ± 0.99[a]	51.34 ± 0.88[a]	53.01 ± 1.27[a]	52.99 ± 1.87[a]	55.63 ± 0.33[a]	0.20 ± 0.28[b]
12 h	48.77 ± 2.83[a]	51.39 ± 0.51[a]	48.53 ± 0.56[a]	49.66 ± 0.24[a]	52.43 ± 0.84[a]	0.00 ± 0.00[b]
24 h	50.67 ± 1.71[a]	46.96 ± 0.21[a]	49.53 ± 1.69[a]	47.45 ± 3.94[a]	52.07 ± 0.19[a]	0.00 ± 0.00[b]

Differences in lowercase letters in a row indicate statistically significant differences between storage media by Tukey' honestly significant difference post hoc test ($p < 0.0034$). HBSS: Hank's balanced salt solution; PDRN: Polydeoxyribonucleotide.

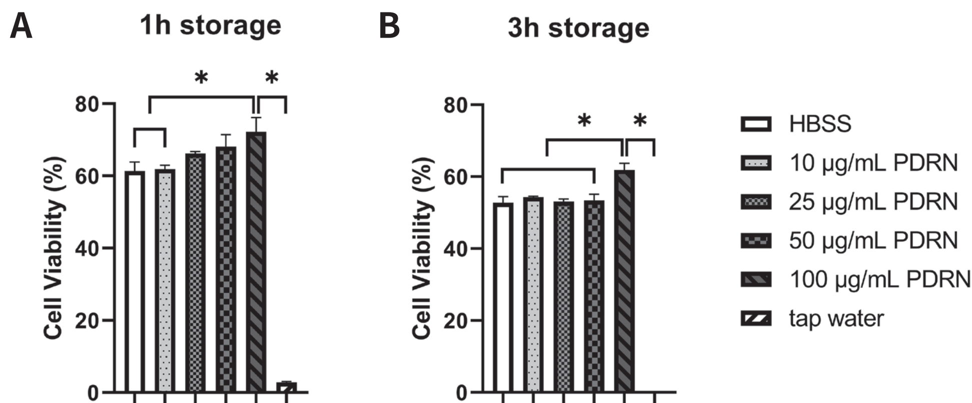

Fig. 4. Graph showing the viability (%) of PDL cells stored in each storage medium for (A) 1 hour and (B) 3 hours using the Live/Dead assay. The data are presented as the mean and standard deviation values.
*$p < 0.0034$, indicating that the difference between groups was statistically significant.
HBSS: Hank's balanced salt solution; PDRN: Polydeoxyribonucleotide.

in tap water were not viable after 1 hour of storage. The number of viable cells in the 50 and 100 µg/mL PDRN solutions was higher than that in the other solutions until 6 hours.

2. Effect of PDRN on NO production

The production of NO in PDL cells stored in HBSS after more than 3 hours of storage was significantly higher than that observed at 0 and 1 hour ($p < 0.0001$, Fig. 6A). A similar increase was observed in cells stored in 50 µg/mL PDRN solution, statistical significance notwithstanding (Fig. 6B). Alternatively, the NO production in cells stored in 100 µg/mL PDRN solution significantly decreased with the increase in storage time until 3 hours ($p < 0.0001$, Fig. 6C).

3. Effect of PDRN on the expression of inflammation-related cytokines

qRT-PCR analysis showed that PDRN significantly reduced the expression levels of inflammation-related cytokines. The expression levels of TNF-α and IL-6 in cells stored in the 50 and 100 µg/mL PDRN solution groups were significantly lower than those in cells stored in the HBSS group ($p < 0.01$, Fig. 7A, C). No significant differ-

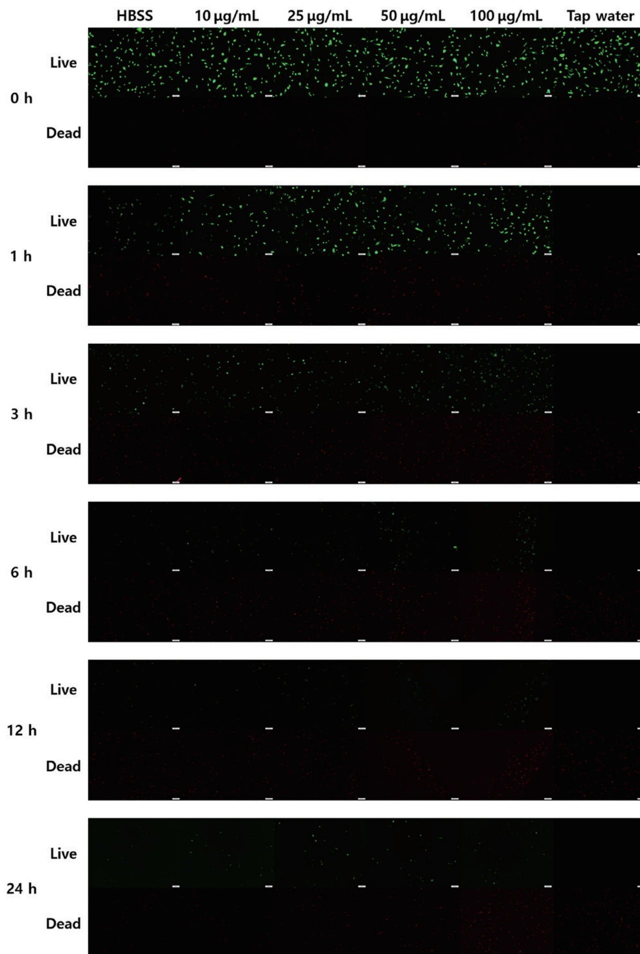

Fig. 5. Fluorescence microphotographs of PDL cells in each storage medium at different periods of time. The live and dead PDL cells exhibited green and red fluorescence, respectively. (100× magnification, scale bar = 100 μm).
HBSS: Hank's balanced salt solution.

ences in the expression levels of TNF-α and IL-6 were seen between cells in the 50 and 100 μg/mL PDRN solutions. The expression levels of IL-4 and IL-10 were decreased in a concentration-dependent manner. IL-4 and IL-10 levels in cells stored in the 50 and 100 μg/mL PDRN solution groups were significantly downregulated compared to those in cells stored in the HBSS (p < 0.01, Fig. 7B, D).

Discussion

An inflammatory reaction is a common complication in the PDL tissues of an avulsed tooth. The inflammatory response of a replanted tooth after delayed replantation is greater than that after immediate replantation. A previous study on inflammatory cytokines and matrix metalloproteinases (MMPs) in replanted teeth found that the expression levels of IL-1β, IL-6, and MMP-1, MMP-2, MMP-7, MMP-8, and MMP-9 were significantly upregulated with the increase in the dry time[4]. Furthermore, another study reported a significant difference in the healing process via TNF-α signaling between teeth that were immediately replanted and those that underwent a delay in replantation[26]. In a retrospective clinical study that evaluated the survival and complications of avulsed and replanted teeth, the probability of tooth loss and in-

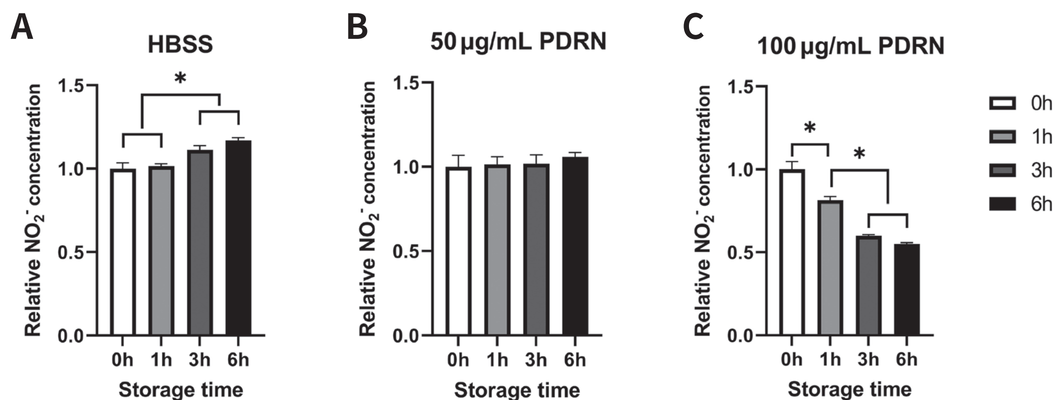

Fig. 6. NO production in (A) HBSS and (B) 50 and (C) 100 μg/mL PDRN solutions. The data are presented as the mean and standard deviation values.
*p < 0.0084, indicating that the difference between groups was statistically significant.
HBSS: Hank's balanced salt solution; PDRN: Polydeoxyribonucleotide.

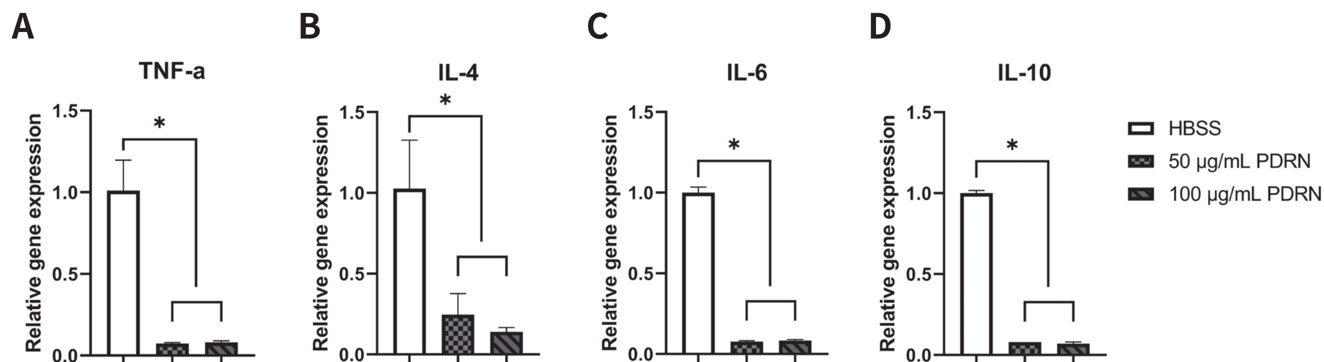

Fig. 7. Relative expression levels of pro-inflammatory cytokines in PDL cells stored in each storage medium for 3 hours. The expression levels of (A) TNF-α, (B) IL-4, (C) IL-6, and (D) IL-10 were analyzed using qRT-PCR. The data are presented as the mean and standard deviation values.
*$p < 0.0167$, indicating that the difference between groups was statistically significant.
HBSS: Hank's balanced salt solution; PDRN: Polydeoxyribonucleotide.

flammatory tooth resorption increased with the increase in the extra-alveolar storage time[27]. It is important to preserve the vital and intact PDL tissues and reduce the inflammatory response in order to prevent inflammatory root resorption[28,29]. Therefore, a storage medium that can ensure the viability of the PDL cells and suppress the inflammatory reaction is required to improve the prognosis of replanted teeth.

PDRN has been reported to enhance tissue regeneration and wound healing through the salvage pathway and via the activation of the adenosine A_{2A} receptor[30,31]. Therefore, it has been widely investigated in the fields of regenerative medicine and biomedical engineering[32]. Additionally, the anti-inflammatory properties and therapeutic effects of PDRN on inflammatory diseases have been studied[33]. Due to these characteristics of PDRN, we thought that it would have a positive effect on the healing of replanted teeth. Thus, PDRN was selected as the subject of subsequent experiments to develop a storage medium for avulsed teeth.

In both the CCK-8 and Live/Dead assays, cells stored in the 100 μg/mL PDRN solution showed the highest cell viability. Especially, in both experiments with 3 hours of storage, 100 μg/mL PDRN showed a significantly higher cell-preserving effect than HBSS. This finding is consistent with those of previous studies. In a study targeting human corneal endothelial cells, PDRN (100 μg/mL) significantly increased the survival rate of cells under oxidative stress[34]. In another in vitro study, PDRN significantly enhanced the proliferation of human skin fibroblasts in a concentration-dependent manner, and the effect was highest at a concentration of 100 μg/mL[35]. Furthermore, 100 μg/mL PDRN in serum-free medium significantly increased the cell viability of RAW 264.7 cells treated with zoledronic acid and LPS[36].

To ascertain whether PDRN has anti-inflammatory effects on PDL cells, a NO detection assay was performed. NO production was significantly increased in PDL cells stored in HBSS after 3 hours of storage. However, the production was significantly decreased in PDL cells stored in 100 μg/mL PDRN solution. These results suggest that PDRN exerts an anti-inflammatory effect on PDL cells by suppressing nitric oxide synthase (NOS). PDRN was reported to inhibit NO production in other human cells[21,31]. An excessive amount of NO can contribute to the destruction of periodontal tissues. Inducible NOS (iNOS) expression and NO production were found to be increased in inflamed periodontal tissues[37-39]. Therefore, PDRN could protect PDL cells from the oxidative damage of NO and reduce the inflammation of periodontal tissues surrounding the replanted tooth.

Additionally, we conducted the qRT-PCR analysis to

evaluate the anti-inflammatory effect of PDRN on PDL cells by identifying the expression levels of inflammatory cytokines. PDRN significantly suppressed the expression levels of TNF-α, IL-4, IL-6, and IL-10. TNF-α is a critical pro-inflammatory mediator in the destruction of periodontal tissues. Additionally, it exerts a significant impact by impeding the migration of PDL cells and inhibiting PDL regeneration[40,41]. IL-6 plays crucial roles in various aspects, including the initiation and progression of inflammation and the resorption of alveolar bone[42]. The findings of the current study suggest that PDRN inhibits the inflammatory response by reducing the expression levels of several inflammatory cytokines. In previous studies, PDRN has been reported to have anti-inflammatory effects by reducing the inflammatory cytokines such as TNF-α, IL-1β, IL-6, and high mobility group box 1, via adenosine A_{2A} receptor signaling[14,33-35,43]. Adenosine is an important modulator of inflammatory function in periodontal tissues[44]. In particular, activation of the adenosine A_{2A} receptor was reported to inhibit the expression of pro-inflammatory cytokines[45]. PDRN, an adenosine A_{2A} receptor agonist, significantly inhibited the inflammatory cascade and the apoptotic reaction in PDL tissues via adenosine A_{2A} receptor stimulation[46]. Thus, storing the avulsed teeth in storage media containing PDRN could enhance the prognosis of replanted teeth by suppressing the inflammatory response of the PDL cells and periodontal tissues.

Furthermore, PDRN can promote angiogenesis by increasing the expression levels of vascular endothelial growth factor (VEGF)[47-51]. The application of VEGF during the replantation of avulsed teeth increased angiogenesis. Therefore, it reduced external root resorption and ankylosis and enhanced the reattachment of the teeth to the surrounding tissues[52]. In addition, topical treatment with VEGF improved neovascularization in the human dental pulp and could be beneficial for the replantation of avulsed immature teeth[53]. Therefore, the PDRN storage medium may be useful in regenerating the pulp tissues and repairing the periodontal tissues in replanted immature teeth.

However, our study has some limitations. Although PDL cells were stored in an environment to imitate the extraoral condition, the dry conditions before transferring the avulsed tooth to the storage medium were not considered in this study. To comprehensively understand the biological effect of PDRN on replanted teeth, it is necessary to investigate the impacts of PDRN on cell cycle regulation, cell proliferation, and angiogenesis. However, our research only focused on evaluating the cell-preserving and anti-inflammatory properties of PDRN. Furthermore, since the scope of this study is solely focused on the effects at the cellular level, it may be challenging to apply the findings directly to clinical situations.

Additional in vitro experiments are needed to evaluate the wound healing and angiogenesis effects of PDRN on PDL cells. Subsequent investigations involving human and animal studies are warranted to validate the findings of the current study.

Conclusion

In conclusion, our study demonstrated that PDRN storage medium could exert cell-preserving and anti-inflammatory effects on the PDL tissues of avulsed teeth.

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Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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국문초록

탈구치 저장 매체로서 치주인대 세포에 미치는 Polydeoxyribonucleotide의 효과에 대한 연구

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본 연구는 탈구된 치아의 저장 매체로서 polydeoxyribonucleotide (PDRN)의 적합성을 평가하고자 하였다. HBSS와 10, 25, 50, 100 µg/mL 농도의 PDRN 용액과 수돗물에 저장된 인간 치주인대 세포의 생존율을 측정하기 위해 Cell Counting Kit-8 assay와 Live/Dead assay를 시행하였다. 또한, PDRN의 항염증 효과를 평가하기 위한 NO 검출 및 qRT-PCR 실험을 진행하였다. 100 µg/mL 농도의 PDRN 용액에 저장된 치주인대 세포의 생존율이 다른 용액보다 유의하게 높았다($p < 0.01$). 또한, 100 µg/mL 농도의 PDRN 용액은 유의하게 NO의 생산을 줄였다($p < 0.0001$). 그리고, HBSS 용액에 비하여 50 및 100 µg/mL 농도의 PDRN 용액에서 유의하게 tumor necrosis factor α, interleukin (IL)-4, IL-6, 그리고 IL-10의 발현이 낮았다($p < 0.01$). 이 연구를 통해 PDRN은 치주인대 세포에 세포 보존 및 항염증 효과를 가진 것으로 밝혀졌다. 이 연구는 효과적인 탈구치 저장 매체의 개발을 위한 향후 추가적인 실험의 기반이 될 수 있을 것이라 생각한다. [J Korean Acad Pediatr Dent 2023;50(3):347-359]

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