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Impact of Exposure to Commonly Used Carbamide Peroxide on Dental Pulp Stem Cells

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Abstract: Background: This study investigated the contact between adult dental pulp stem cells (DPSCs) and carbamide peroxide (CP), a bleaching agent that is a popular choice for at-home whitening products, using an in vitro model. **Objectives**: The aim of this study was to evaluate the impact of exposure to different concentrations and timings of a commonly used peroxide-based home tooth-whitening product on DPSCs. **Materials and methods**: Human DPSCs obtained from impacted third molars were cultured and exposed to various concentrations of carbamide peroxide (0.1%, 0.5%, and 1%). The effects of CP on DPSC proliferation and apoptosis were investigated by MTT assay and flow cytometry. Migration was investigated by micrographs of wound healing. An enzyme-linked immunosorbent assay (IL-6 and IL-8) was used to investigate the CP-stimulated cytokine production of DPSCs. Each experiment was performed three times with independent batches of DPSCs. Statistical analysis of the collected data was performed using one-way and two-way ANOVAs with the significance threshold set at $p < 0.05$. Tukey's post hoc multiple comparison test was used to identify differences between groups. **Results**: Cell viability and adherence were lower in the CP-exposed cells compared to the non-stimulated cells, probably due to increased cell death (** $p \leq 0.01$, **** $p \leq 0.0001$). CP-stimulated DPSCs exhibited a dose-dependent release of IL-6 and IL-8 (**** $p \leq 0.0001$). CP did not affect wound healing at any concentration tested. **Conclusions**: Human DPSCs were able to sense CP. Consequently, CP contributed significantly to cell apoptosis and local inflammatory responses through cytokine release.

Keywords: DPSCs; carbamide peroxide; apoptosis; inflammatory reaction

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1. Introduction

Aesthetics are philosophical conceptions of beauty that are given high value in modern Western society. In today's society, there is an overwhelming tendency for individuals to project themselves in an optimal manner. Social science research underscores the substantial influence of physical appearance on life experiences, emphasizing its central role as a reflection of individual character. It is human nature to attribute positive personality characteristics to attractive individuals, and this often leads to advantages in various areas of life, such as career opportunities, social status, and romantic relationships [1].

Dentistry has evolved from a primarily medical service to an elective aesthetic service, often at the patient's request [2]. The appearance of teeth has a significant impact on social perceptions; studies show that people with straight, white teeth are seen as more attractive and confident [3]. In addition, there is strong evidence that facial and dental aesthetics significantly influence social judgments, with even children and adolescents evaluating physical attractiveness, including dental appearance, in their social choices [4,5].

This attitude can often persist into adulthood, where individuals may continue to prioritize physical appearance in their personal and professional relationships [6].

In dentistry, tooth whitening has become an increasingly popular cosmetic treatment, with many patients seeking to improve the appearance of their teeth for aesthetic reasons. This trend is reflected in the growing interest in tooth-whitening treatments in recent years [7].

The most common ingredients used in bleaching are carbamide peroxide and hydrogen peroxide, which are used at various concentrations [8].

Carbamide peroxide is a slower-acting bleaching agent than hydrogen peroxide, and it releases hydrogen peroxide over a longer period of time [9]. This makes it a popular choice for at-home whitening products because it causes less sensitivity than the high-concentration hydrogen peroxide formulations used in dental offices.

A 2018 Cochrane review assessed the safety and efficacy of home-based tooth-whitening products. It reported that tooth sensitivity and oral irritation were the most common adverse effects of these products and that these effects were generally mild and transient [10].

Both the organic and inorganic components of the tooth structure undergo significant changes due to the chemical properties of bleaching agents. These changes result in morphological alterations such as increased permeability and surface roughness, which can weaken the tooth's mechanical integrity. Furthermore, bleaching agents can trigger molecular transformations upon reaching the dental pulp, leading to oxidative stress within pulp cells and the subsequent release of pro-inflammatory substances. While bleaching treatments are effective in achieving the desired results, tooth sensitivity remains a prominent side effect. In particular, prolonged exposure to bleaching agents at lower concentrations tends to have more harmful effects on tooth structure [11].

Therefore, it is important to investigate the potential impacts of common dental treatments on dental pulp stem cells, as these cells play an important therapeutic role in maintaining the health and vitality of teeth. By evaluating the impacts of various concentrations of peroxide-based at-home tooth-whitening products on dental cells, including dental pulp stem cells (DPSCs), this study aimed to provide valuable insights into the potential effects of these products on the dental pulp stem cell reservoir.

The use of an enamel–dentin or dentin disc model provided a realistic and relevant environment for studying the impacts of peroxide-based tooth-whitening products on DPSCs, as it allowed for the direct and indirect exposure of these cells to the products in a controlled and standardized manner.

Overall, the findings of this study could have important implications for the use of peroxide-based tooth-whitening products and their potential impact on dental health.

The aim of this study was to evaluate the impact of exposure to different concentrations and timings of a commonly used peroxide-based home tooth-whitening product on DPSCs.

2. Materials and Methods

The authors followed a protocol for the isolation of dental pulp stem cells (DPSCs) and analysis of flow cytometry, cell viability, adhesion, and other assays that was previously described [12].

All flow cytometry analysis was conducted on a BD LSRFortessa™X-20 (BD Biosciences, Piscataway, NJ, USA), and the data were analyzed by FlowJo LLC software (version 9.7.7, Ashland, OR, USA).

2.1. Isolation and Culture of DPSCs

The procedure used to extract healthy human teeth and dental pulp tissue in this study was approved by the Ethics Committee of the Children's Hospital of Queen Fabiola, Free University of Brussels. Patients/parents provided their written informed consent. Pulp tissue was obtained from impacted third molars collected from healthy subjects that were 13–14 years of age (n = 3). Coronal pulp was retrieved and minced into small fragments before digestion in a solution of 3 mg/mL collagenase type I (Gibco Life Technologies, Carlsbad, CA, USA) and Alpha Minimum Essential Media (α-MEM Modification, Capricorn Scientific, Ebsdorfergrund, Germany) for 1 h at 37 °C in 5% CO_2. The cell suspension

was filtered through a 70 µm cell strainer (Greiner Bio-One GmbH, Frickenhausen, Germany) and pelleted. Dental pulp cells were seeded into 25 cm^2 cell culture flasks (Cellstar®, Greiner Bio-One GmbH) and cultured in α-MEM (Capricorn Scientific) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, St-Louis, MO, USA), 0.5% 10,000 mg/mL penicillin/streptomycin (Lonza, Walkersville, MD, USA), and 1% 100× Glutamax™ (Gibco Life Technologies) at 37 °C in 5% CO_2. The medium was changed every 2–3 days. When the cells became sub-confluent, they were collected by trypsinization (0.5% trypsin and EDTA 1x, Gibco Life Technologies) and subjected to subsequent passages. DPSCs from passages 2 to 4 were used in this study.

2.2. Sample Preparation

2.2.1. Bleaching Agent

One syringe of Opalescent 16%PF (Ultradent, South Jordan, UT, USA) was diluted in 15 mL of medium (100 mg/mL) and maintained at 37 °C for 6 h in 5% CO_2. Next, the supernatants were collected and sterilized. Aliquots of 0.1, 0.5, and 1% were prepared and stored at −20 °C until they were used.

2.2.2. Tooth Samples

Dentin discs were prepared from impacted third molars. The vestibular and lingual/palatal surfaces of each tooth were sectioned using a disc positioned near or in contact with the pulp chamber. Blocks measuring 5 mm × 5 mm × 4 mm were then prepared, considering that the mesiodistal diameter of a tooth ranges from 7 to 9 mm. A central cavity measuring 2 mm × 2 mm × 2 mm was then prepared on the dentinal surface (Figure 1), and the dentin surface was treated with 0.5 M EDTA in PBS for 30 s to remove the smear layer and sterilized in an autoclave. The dentin thickness at the bottom of the cavity was 2 mm between the bottom of the cavity and the pulp-side surface of the block. All measurements were made using a digital caliper and a digital height gauge.

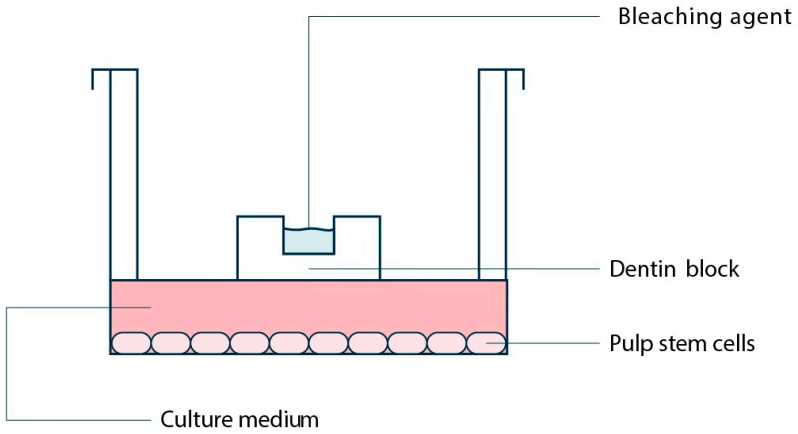

Figure 1. Transwell system applied to assess cell viability.

In order to eliminate any cracks that occurred during tooth preparation, the dental samples were tested with bidistilled water in their cavities and stored in bidistilled water until they were used. The samples were randomly divided into four groups (n = 5).

2.2.3. Flow Cytometry

DPSC marker analysis: A total of 5 × 10^6 cells were harvested using 0.5% trypsin and EDTA, followed by resuspension in a fluorescence-activated cell-sorting buffer solution (PBS supplemented with 0.1% BSA, 0.02% azide, and 1 mM EDTA; BD Biosciences). The cells were characterized using a human MSC analysis kit (BD Biosciences) according to the manufacturer's protocol. The kit included an MSC-positive antibody cocktail (FITC mouse Anti-Human CD90, APC Mouse Anti-Human CD73, and PerCP-Cy5.5 Mouse Anti-Human

CD105) and an MSC-negative antibody cocktail (PE CD34, PE CD45, PE CD11b, PE CD19, and PE HLA-DR).

Apoptosis analysis: DPCSs were seeded in 25 cm^2 flasks (Greiner Bio-One GmbH) at a density of 1×10^6 cells and allowed to reach full confluence. Then, the samples were incubated in 10 mL of media containing different concentrations of the bleaching agent. After 3, 6, and 24 h, the cells were trypsinized, centrifuged, and resuspended in the media. The cells were double-stained with PE-conjugated Annexin-V and 7-AAD (Guava Nexin Reagent, Guava Technologies, Hayward, CA, USA). The percentages of live (Annexin-V-/7-AAD-), early apoptotic (Annexin-V+/7-AAD-), late apoptotic (Annexin-V+/7-AAD+), and necrotic cells (Annexin-V-/7-AAD+) were determined using flow cytometry.

2.2.4. Cell Viability Assay

I. DPSCs were plated at a density of 1×10^4 cells per well with a volume of 100 µL in a 96-well plate (Greiner Bio-One GmbH). The cells were grown in a cell medium culture and allowed to adhere for 24 h at 37 °C in 5% CO_2. The samples were incubated in media containing different concentrations of the bleaching agent. Cell viability was evaluated by the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) to a purple product by cellular mitochondria. At 1, 3, 6, and 24 h post-stimulation, cells were washed with sterile PBS and incubated with 0.5 mg/mL (100 µL/well) MTT (VWR, Amresco, OH, USA). Following a one-hour incubation, the supernatant was removed from the plates and isopropyl alcohol (100 µL/well) was added. The absorbance of the MTT was measured using a spectrometer (Multiscan Ascent 354 microplate reader, Labsystems, Vantaa, Finland) at 540 nm.

II. DPSCs were plated at a density of 1×10^5 cells per well with a volume of 1 mL in a 24-well plate (Greiner Bio-One GmbH). The cells were grown in a cell medium culture and allowed to adhere for 24 h at 37 °C in 5% CO_2. Cell culture inserts with 8 µm pores (Thincert™, Greiner Bio-One GmbH) were used in this experiment. The dentin disc cavities were filled with the bleaching agent and were then placed in the Transwell insert to stimulate the DPSCs with the bleaching agent (Figure 1). At 1, 3, 6, and 24 h post-stimulation, the Transwell insert was removed and the cells were washed with sterile PBS and incubated with 0.5 mg/mL (100 µL/well) MTT (VWR, Amresco, OH, USA). Following a one-hour incubation, the supernatant was removed from the plates and isopropyl alcohol (100 µL/well) was added. Finally, the medium was transferred to an optical 96-well plate (Greiner Bio-One GmbH), and the absorbance of the MTT was measured using a spectrometer (Multiscan Ascent 354 microplate reader, Labsystems) at 540 nm.

2.2.5. Wound-Healing Assay

In total, 2×10^5 DPSCs were seeded into a 6-well plate and allowed to reach full confluence. A wound was inflicted by making a scratch through the cell monolayer with a 200 µL pipette tip. Three parallel vertical lines were scratched on the bottom of each well (Greiner Bio-One GmbH) to create nine intersections. After wounding, the cells were washed with PBS to remove debris and incubated with cell culture media with different concentrations of the bleaching agent for 24 h. Microphotographs of the scratch were taken at 0, 12, 18, and 24 h post-wounding. The cultured cells were observed with a phase-contrast microscope (Olympus, Tokyo, Japan). The wound closure rate was determined at each intersection by image analysis using Image J version 1.54g (National Institute of Health, Bethesda, MD, USA).

2.2.6. Adhesion Assay

Ninety-six-well plates (Greiner Bio-One GmbH) were coated with 50 µL of type I collagen (40 mg/L in a 1× PBS dilution) (Collagen rat tail, Roche Diagnostics GmbH, Mannheim, Germany) overnight at 4 °C. The wells were washed twice with 1× PBS to remove unbound collagen and blocked with 1% BSA for 1 h at 37 °C in 5% CO_2. Then, 1×10^4 cells were added to each well in the presence of different concentrations of the

bleaching agent. The cells were incubated for 90 min at 37 °C in 5% CO_2. The wells were then washed three times with 1× PBS. The cells were fixed with 200 µL of 4% paraformaldehyde per well at room temperature for 15 min and washed three times with 1× PBS. The cells were stained with 100 µL of 0.1% crystal violet per well for 10 min at room temperature. The wells were washed three times with ddH_2O prior to the addition of 100 µL of 10% acetic acid to each well. The optical density was measured using a spectrometer (Multiscan Ascent 354 microplate reader, Labsystems) at 595 nm.

2.2.7. Enzyme-Linked Immunosorbent Assay

In total, 2×10^5 DPSCs per well were seeded in a 6-well plate and allowed to reach full confluence. Then, the samples were incubated in 3 mL/well, with the media containing different concentrations of the bleaching agent. At 6, 12, and 24 h post-stimulation, the supernatants were collected and stored at -80 °C until assays were performed. The levels of IL-6 and IL-8 (CX-CL8) in the culture supernatants were analyzed using commercially available ELISA kits according to the manufacturer's protocol (Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA). Briefly, 96-well plates (Thermo Fisher Scientific) were coated overnight at 4 °C with a capture antibody diluted 250 times in PBS. The plates were washed three times with a wash buffer and blocked for 1 h with ELISA/ELISPOT Diluent. The plates were then washed once before the manufacturer's standards and the samples were added to the plates for 2 h. After washing 3–5 times, the plates were incubated with a detection antibody for one hour. The plates were washed 3–5 times in total and were incubated with streptavidin–horseradish peroxidase for 30 min. After washing 5–7 times, the plates were incubated with a substrate solution (TMB) for 15 min. At the end, a stop solution was added, and the optical density was measured using a microplate reader (Multiskan Ascent 354 microplate reader, Labsystem) at 450 nm.

2.3. Statistical Analysis

The results were expressed as mean values ± Standard Deviations (SDs) obtained from at least three independent experiments. Each assay was repeated three times on independent batches of DPSCs. The data collected were analyzed statistically using one-way and two-way ANOVAs. The level of significance was set at $p < 0.05$. Tukey post hoc multiple comparison tests were carried out to determine the differences between groups. The statistical analyses were performed using GraphPad Prism version 9.4.1 (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1. Characterization of Adult DPSCs

Common mesenchymal stem cell markers (CD73, CD90, and CD105) were consistently positive, whereas hematopoietic markers (CD34, CD45, CD11b, CD19, and HLA-DR) were negative in all samples. The multipotency of the DPSCs was evaluated through incubation with osteogenic, chondrogenic, and adipogenic differentiation media. After three weeks of differentiation, the differentiated DPSCs showed the ability to secrete a calcified matrix or large amounts of proteoglycans or to accumulate intracellular lipids (Figure 2A,B)

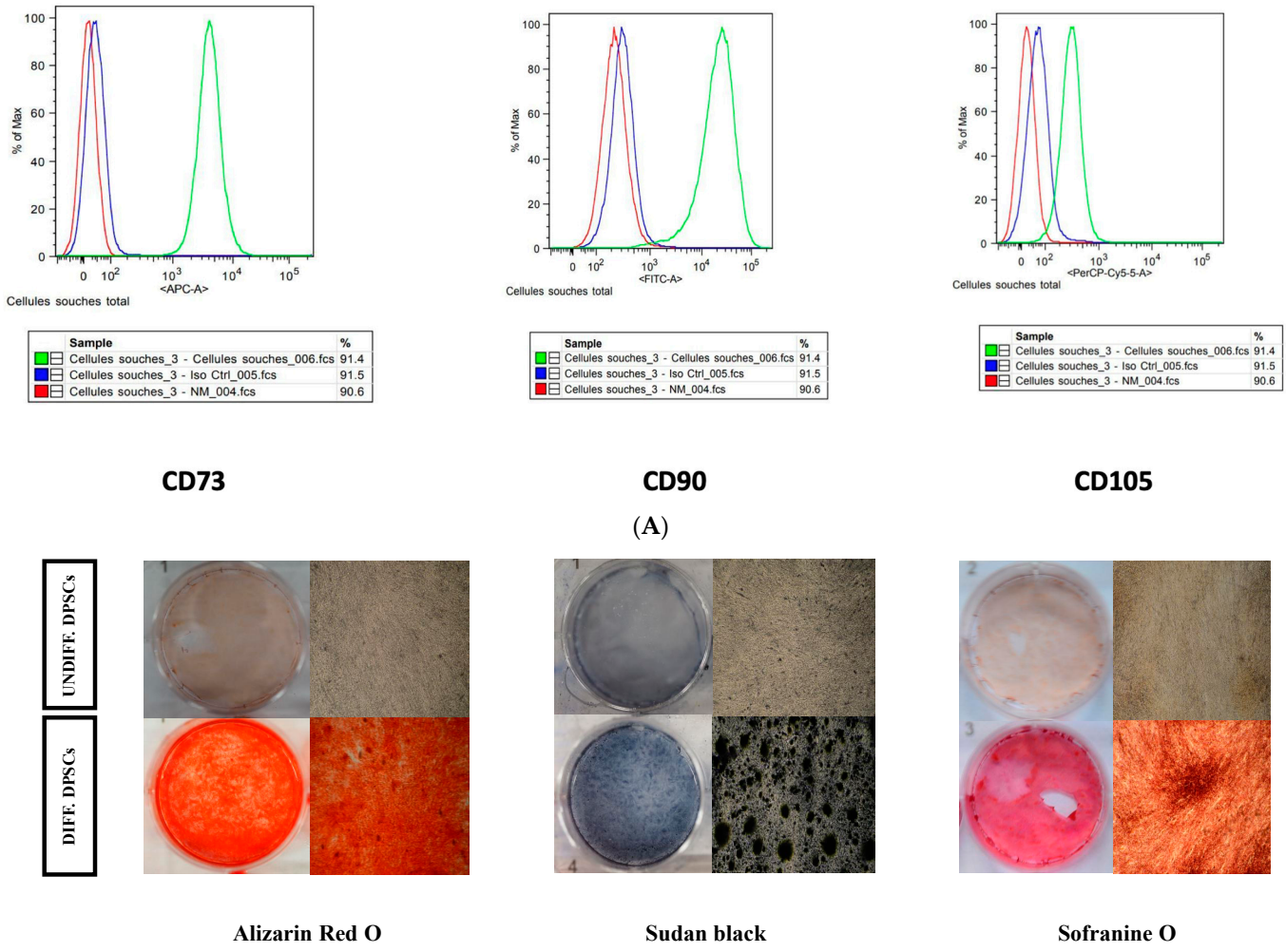

Figure 2. Phenotypic characterization of DPSCs. (**A**) Flow cytometric analysis confirmed the expression of the mesenchymal stem cell markers CD73, CD90, and CD105 by DPSCs. (**B**) After 3 weeks of exposure to respective differentiation media, DPSCs were able to commit towards osteogenic (Alizarin Red), adipogenic (Sudan black), or chondrogenic (Safranine O) lineages.

3.2. Bleaching Agent Decreased DPSC Proliferation and Induced Cell Death

I. Incubation for 3 h or 24 h with the bleaching agent decreased the proliferation of the DPSCs, with the lowest proliferation recorded for 1% at 24 h ($p < 0.0001$) (Figure 3A).

II. Induction with the bleaching agent decreased the proliferation of the DPSCs significantly ($p < 0.0001$) within the first hour of incubation (Figure 3B).

As the number of cells per well was also a function of the cell death rate, the production of viable cells and the numbers of cells entering early apoptosis, late apoptosis, and necrosis were evaluated. Each concentration of the bleaching agent significantly induced ($p < 0.005$, $p \leq 0.0001$) the early apoptotic phase in the DPSCs (Figure 4).

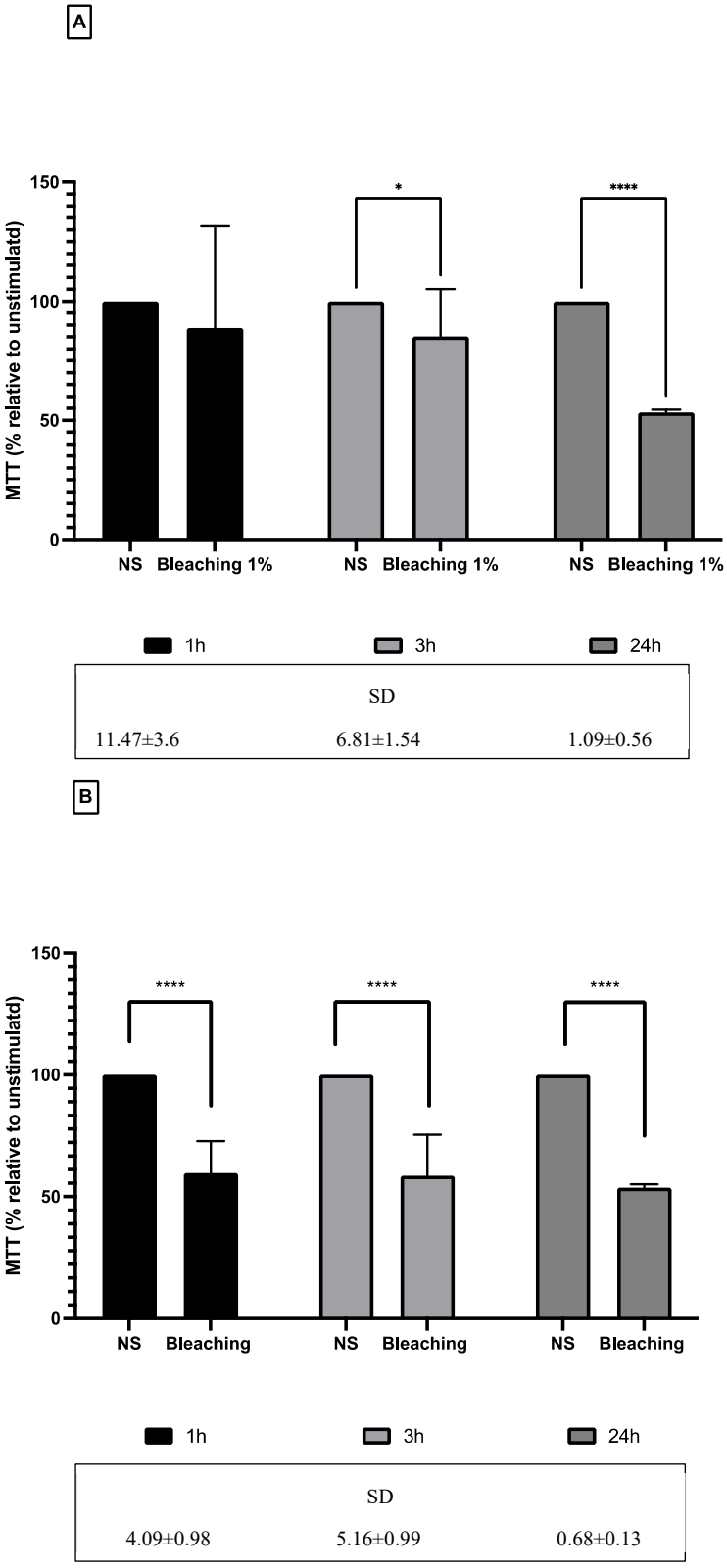

Figure 3. Viability assessment of DPSCs after bleaching agent stimulation. (**A**) Exposure to the bleaching agent for 3 h significantly decreased the number of living cells in the wells. Exposure to the bleaching agent for 24 h significantly decreased the number of living cells in the wells by 45% (* $p < 0.01$, **** $p \leq 0.0001$). (**B**) Exposing the dentin disc to the bleaching agent for 1 h significantly decreased the number of living cells in the wells (**** $p \leq 0.0001$).

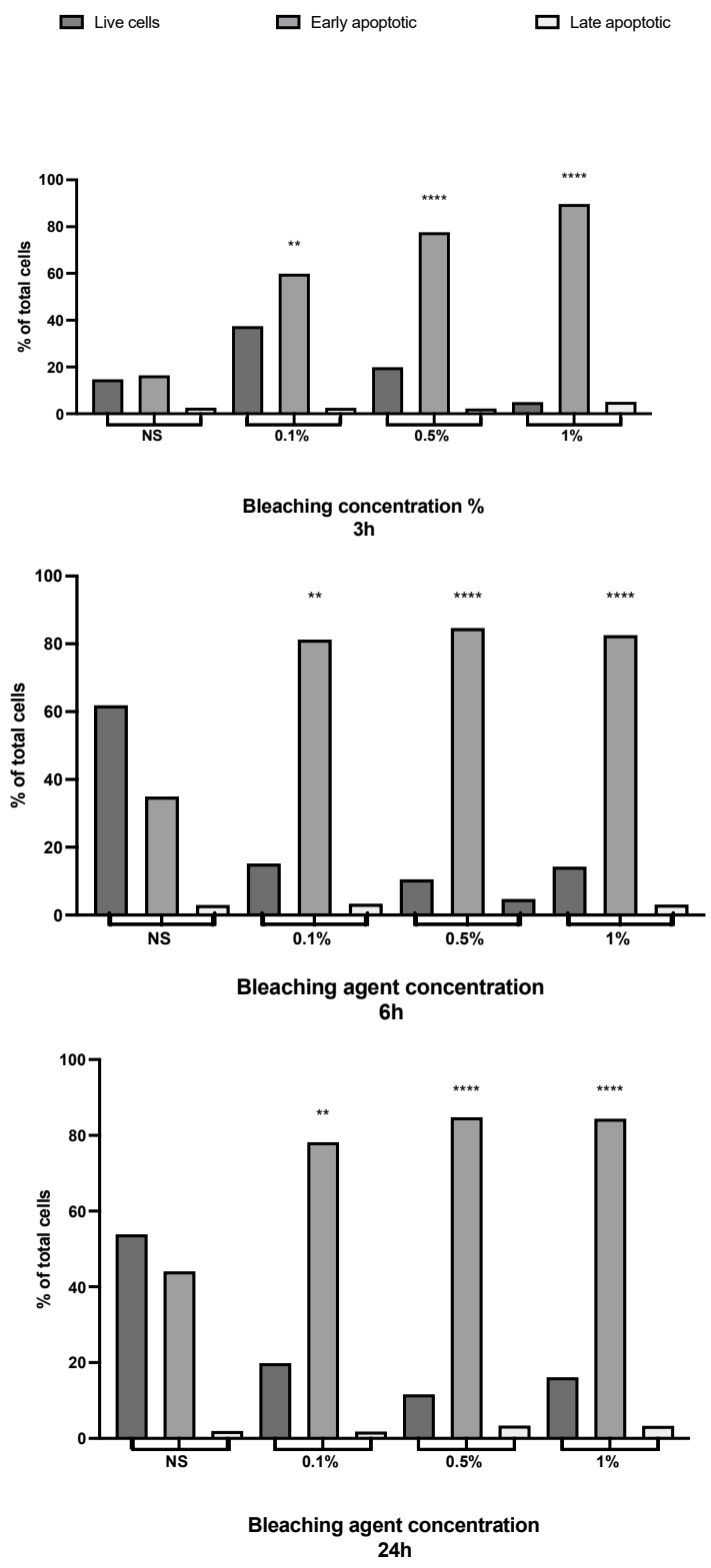

Figure 4. Viability assessment and cell death. Proportion of living cells (%) was quantified by flow cytometric expression of markers Annexin-V and 7-AAD. Annexin-V- and/or 7-AAD-negative cells were considered living cells, Annexin-V-positive and 7-AAD-negative cells were considered early apoptotic cells, and 7-AAD-positive cells were considered late apoptotic or necrotic cells. Stimulation for 3 h induced a significant number of apoptotic cells compared to the unstimulated condition. Data are presented as means ± SDs. ** $p \leq 0.01$, **** $p \leq 0.0001$. Each graph illustrates one representative experiment out of three independent experiments.

3.3. Bleaching Agent Influenced Cell-to-Matrix Adhesion but Did Not Modulate Horizontal Migration

Treatment with the 0.5% and 1% concentrations significantly decreased the numbers of adherent cells compared with the unstimulated group ($p < 0.05$) (Figure 5). Cells were exposed to increasing concentrations of the bleaching agent, which did not modulate horizontal migration. However, the cells exposed to the 1% concentration showed a slowing of wound closure compared with the unstimulated group (Figure 6).

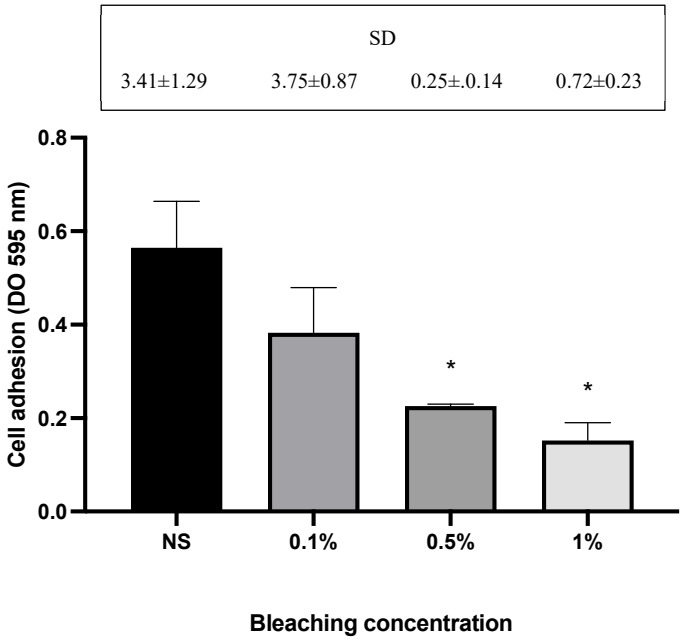

Figure 5. Effect of bleaching agent on adhesion of DPSCs. Exposure to various concentrations of bleaching agent significantly decreased cell adherence to collagen matrix compared with unstimulated group (* $p < 0.01$).

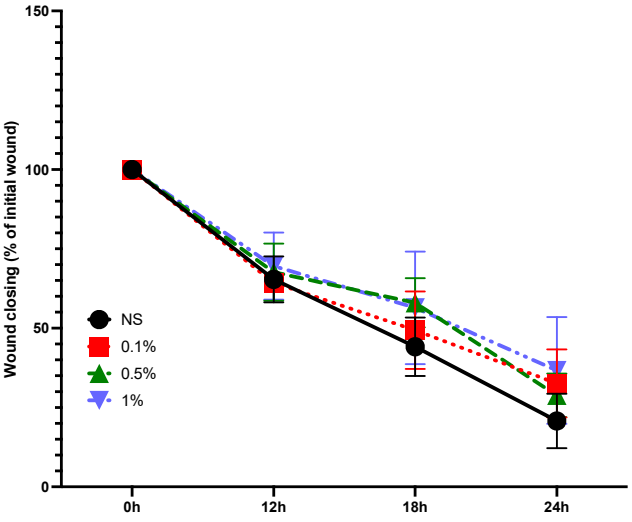

Figure 6. Effect of the bleaching agent on the migration of DPSCs. Horizontal migration was evaluated using a wound-healing assay of confluent monolayers. The relative cell migration rate in each group was calculated based on the closure of the initial gap and was expressed as the % of wound closing. Various concentrations of the bleaching agent did not modulate horizontal migration. However, the cells exposed to the 1% concentration showed a slowing of wound closure compared with the unstimulated group. This graph illustrates one representative experiment out of three independent experiments.

3.4. Bleaching Agent Induced Cytokine Production in DPSCs

Over time, after incubation with different concentrations of the bleaching agent, the production of IL-6 and IL-8 ($p \leq 0.0001$) increased in a dose-dependent manner (Figure 7A,B). This effect was similarly observed at 3 and 24 h. The treatment with the 1% concentration significantly enhanced the production of cytokines.

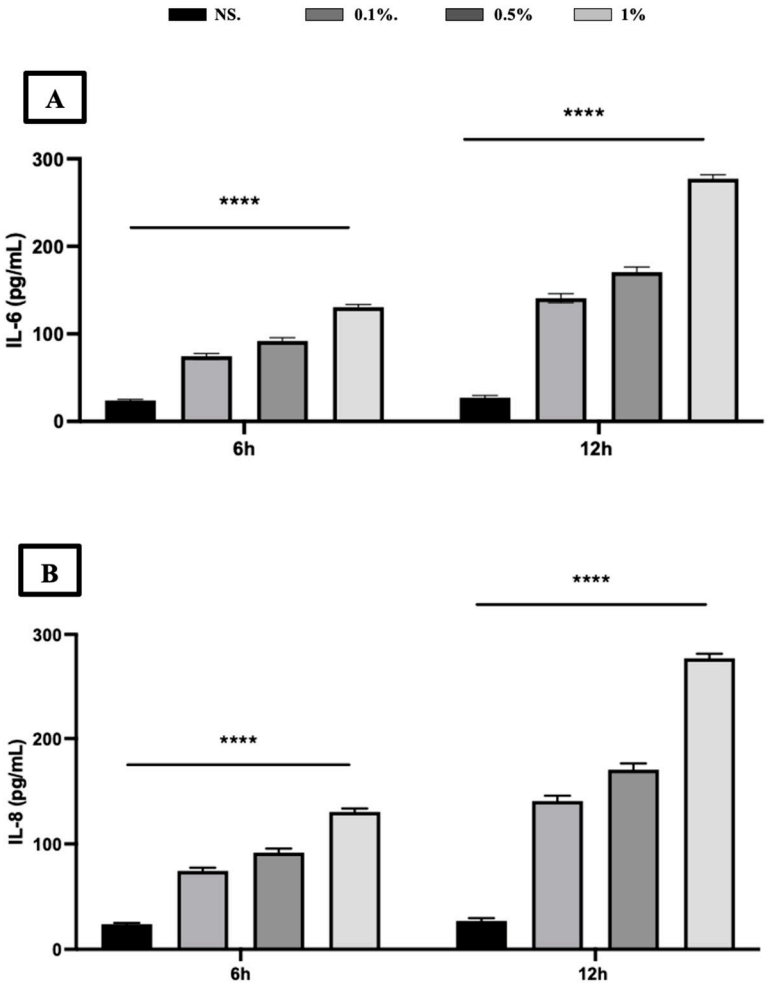

Figure 7. Effect of the bleaching agent on cytokine release. The cytokine content was assessed by ELISA in the supernatant of an in vitro culture of DPSCs stimulated for 6, 12, and 24 h with various concentrations of the bleaching agent. Over time, after incubation with different concentrations of the bleaching agent, the production of IL-6 (**A**) and IL-8 (**B**) increased in a dose-dependent manner (**** $p \leq 0.0001$). Each graph illustrates one representative experiment out of three independent experiments.

4. Discussion

DPSCs represent a small fraction of the resident cells in the dental pulp that remain quiescent under normal conditions. Upon injury or infection, these cells can rapidly activate and migrate to areas with damaged pulp [13]. Through their ability to differentiate into odontoblasts, DPSCs play a critical role in the formation of reparative dentin and are also key regulators of the pulpal immune response [14]. This study aimed to test the null hypothesis that the bleaching agent does not influence diffusion and does not have biological effects on DPSCs.

Carbamide peroxide (CP) is a bleaching agent commonly used in dentistry for vital at-home tooth whitening. CP is a combination of hydrogen peroxide and urea. When applied to teeth, it breaks down into hydrogen peroxide (H_2O_2) and urea. The H_2O_2 dissociates into water and highly reactive oxygen species, including free radicals such as

hydroxyl radicals (OH-), and could reach the pulp chamber, mostly by diffusion through dentinal tubules [15].

In our study, it was shown that there was a relationship between the concentration of the bleaching agent and its cytotoxic effect on DPSCs. As the concentration of the bleaching agent increased, so did its cytotoxic effect. Several studies have investigated the cytotoxic effects of bleaching agents on pulp cells and human gingival fibroblasts. Their results showed that increasing concentrations of a bleaching agent resulted in higher levels of cytotoxicity [16,17]. These consistent findings across multiple studies provide strong evidence for a relationship between the bleaching agent concentration and cytotoxicity and suggest that this relationship may be a general phenomenon rather than a specific characteristic of any particular bleaching agent or experimental design. The findings of the current study support the previous findings [18,19]. This information could be useful in a variety of applications, including the development of safer and more effective bleaching agents. However, it is important to note that the specific concentration at which cytotoxicity occurs may vary depending on the particular cells or tissues being exposed. Overall, our findings suggest that careful consideration should be given to the concentrations of bleaching agents used in different applications to minimize potential cytotoxic effects while still achieving the desired results.

An Annexin apoptosis assay and flow cytometry were used to detect cells undergoing apoptosis, and the results of groups treated with different concentrations of a bleaching agent were compared to a control group. This study found that by the third hour of bleach application, the relative percentage of the inflammatory response was significantly higher in all bleach-treated groups compared to the control group.

To mitigate the inflammatory and apoptotic responses induced by different concentrations and exposure durations of carbamide peroxide, it is essential to explore combinatorial approaches. This investigation could focus on strategies such as the co-administration of, for example, anti-inflammatory agents or growth factors to create a more favorable microenvironment that promotes regenerative processes [20,21]. By comprehensively evaluating these combinatorial approaches, we will aim to improve the efficacy and safety of carbamide peroxide applications, ultimately advancing our understanding of regenerative therapies in the context of dental treatment. In future studies, it could be interesting to elucidate the intricate molecular mechanisms that govern the response of DPSCs to carbamide peroxide, a crucial aspect in understanding the potential applications of this compound in regenerative medicine. This investigation could meticulously unravel the underlying signaling pathways and elucidate the dynamic gene expression profiles that orchestrate the intricate response of DPSCs to carbamide peroxide exposure.

In addition, we used two cell viability models in our study to compare the effects of the bleaching agent at different concentrations. Both models produced similar results, but the results obtained with the dentin discs, which more closely simulated the clinical conditions, showed greater levels of damage than those obtained with arbitrarily chosen concentrations. This suggests that the concentrations of the bleaching agent released through the dental tissues to the pulp are more significant in determining its cytotoxic effect than the concentrations arbitrarily chosen for our study. In addition, the viability and proliferation of the DPSCs significantly decreased within the first hour after bleaching.

The increasing prevalence of adolescents pursuing tooth-whitening treatments has raised concerns regarding the safety and suitability of subjecting this age group to such procedures. The bleaching policy of The American Academy of Pediatric Dentistry advocates for the cautious application of bleaching treatments in adolescents and emphasizes that these procedures should be conducted under the supervision of a qualified dentist [22]. European Communities Directive 2011/84/EU mandates that hydrogen peroxide cannot be used in concentrations exceeding 0.1% for treatments involving patients under the age of 18 [23].

Because of the cytotoxic effects of tooth-whitening products, their use in adolescents, especially those with developing teeth, should be carefully limited to specific cases such as

amelogenesis imperfecta [24], fluorosis [25], and other enamel defects. Tooth whitening should not be used during mixed dentition. Therefore, it is crucial to approach the whitening of teeth in young people with an individualized, conservative strategy that focuses first and foremost on the health and safety of the teeth.

One of the strengths of our study was that it investigated cellular recovery and the host immune response after exposure to H_2O_2-derived free radicals, as this provided important insights into the mechanisms underlying the pulp response to oxidative stress. On a concentration basis, CP appears to be immunogenic. Accordingly, this report indicates that CP is a virulence factor and that DPSCs are able to sense H_2O_2-derived free radicals and induce the secretion of IL-6 and IL-8.

Furthermore, we could explore the multifaceted interplay between oxidative stress, inflammation, and regenerative capacity in the context of DPSCs. By examining these interrelated processes, we will aim to identify and characterize potential targets for modulation and gain valuable insight into the modulation of DPSCs' behavior in the presence of carbamide peroxide. This holistic approach is expected to contribute to a more comprehensive understanding of the molecular dynamics involved and to foster progress in the development of therapeutic strategies that harness the regenerative potential of DPSCs.

The adhesion of the DPSCs to the collagen matrix was affected by the concentration of the bleaching agent; as the concentration of the bleaching agent increased, the adhesion of the DPSCs to the collagen matrix decreased.

The findings of our study help us to understand how DPSCs behave after indirect/direct contact with carbamide peroxide as a tooth-bleaching agent and may serve as a catalyst for the development of novel strategies to enhance the regenerative properties of DPSCs despite an inflammatory environment.

The translation of research findings into clinical applications is essential for the advancement of tooth-bleaching techniques. This process should integrate the regenerative potential of pulp cells, especially DPSCs, into improved bleaching protocols. Researchers must focus on practical implementation in dental clinics to improve both treatment outcomes and the patient experience. The goal is to develop methods that not only effectively whiten teeth but also promote dental tissue health. Such a translational approach will ensure that innovations in materials and scaffolds directly improve the practice of tooth whitening, potentially leading to breakthroughs in dental regenerative medicine and ensuring the safety and effectiveness of new treatments.

5. Conclusions

Our study investigated the effects of carbamide peroxide, a commonly used bleaching agent, on dental pulp stem cells (DPSCs). Our results clearly demonstrate that exposure to carbamide peroxide results in significant cytotoxic effects as early as three hours after application. Specifically, we observed a significant increase in apoptosis and a decrease in cell viability, indicating that carbamide peroxide compromises the cellular integrity and functionality of DPSCs.

Furthermore, DPSC adhesion was found to decrease in a concentration-dependent manner, highlighting the sensitivity of these cells to oxidative stress induced by carbamide peroxide. These findings have significant implications for clinical practices involving tooth bleaching, suggesting that lower concentrations and shorter exposure times may be required to minimize adverse effects on dental pulp tissues.

This study contributes to a deeper understanding of the cellular mechanisms affected by tooth-bleaching agents and highlights the need for further research to optimize safe clinical protocols.

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administration, A.S., I.V., M.B. and N.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The procedure to retain impacted third molars was approved by the Ethics Committee of the Children's Hospital of Queen Fabiola, Free University of Brussels (CEH n° 30/16).

Informed Consent Statement: Patients/parents gave written informed consent.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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