# UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

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Präventive Zahnmedizin und Zahnerhaltung

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## Cultivation of Cryopreserved Human Dental Pulp Stem Cells - A New Approach to Maintaining Dental Pulp Tissue

zur Erlangung des Grades eines Doktors der Zahnmedizin
an der Medizinischen Fakultät der Universität Hamburg.

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## 1. Introduction

Human dental pulp stem cells (hDPSCs) are derived from the ectoderma, which are a type of mesenchymal stem cells (MSCs) that have been extensively studied in recent years. They are considered promising candidates in the field of tissue engineering because of their strong self-renewal, proliferation and multidirectional differentiation abilities into varies types of cells[1], including neuronal cells, osteoblasts, adipocytes and vascular endothelial cells[2]. In addition, several studies have shown that hDPSCs have unique advantages as seed cells for tissue engineering. For example, hDPSCs have a higher proliferative capacity and higher expression of angiogenesis-related genes compared to adipose stem cells[3]. Besides, they have higher proliferative capacity and differentiation potential compared to bone marrow cells[4,5], and had stronger neurotrophic protein expression levels in neuronal regeneration[6].

In general, the freezing of primary hDPSCs is divided into several steps: tooth extraction, pulp isolation, cell extraction and freezing in order to facilitate ready access[7]. However, due to limited conditions, dental clinics usually cannot guarantee a sterile environment of biolab grade; in addition, longer in vitro exposure can easily lead to the death of hDPSCs due to the loss of blood supply to the organism after tooth extraction. Therefore, the longer the in vitro exposure, the higher the risk of hDPSCs apoptosis and being infected. In addition, it is worth noting that the quality and biological properties of MSCs may decrease with increasing donor age, which has been confirmed by a related study[8]. Hence, targeted cryopreservation of discarded

teeth, including third molars, under the conditions of young and healthy donors can ensure, to some extent, that the biological performance of stem cells is at an optimal stage.

With these considerations in mind, in this experiment we chose healthy third molars from young patients and used a novel method to cryopreserve the whole teeth at -80 °C. This method has several advantages compared to conventional cell cryopreservation methods. First, this method does not require the use of liquid nitrogen, which effectively reduces the cost of freezing. Second, freezing immediately after tooth extraction reduces the exposure time of dental tissues outside the body, thus reducing the risk of infection and apoptosis of hDPSCs. In addition, the screening of donor age can selectively ensure the optimal viability and biological properties of hDPSCs, creating good conditions for patients to apply hDPSCs for stem cell therapy in the future. So, this study aims to optimize the freezing and storage method of hDPSCs, to ensure the stem cell performance while reducing the risk, and to provide a theoretical basis for the clinical application of hDPSCs.

# 2. Material and Methods

### *2.1. Collection of Third Molars*

Eighteen healthy third molars requiring clinical extraction from young people aged 15-19 years were collected. The extracted teeth were immediately placed in DMEM and transferred to the laboratory, after which they were soaked in DPBS solution containing antibiotics for 30-60 minutes. The experimental procedure is shown in the

flow chart in Figure 1.

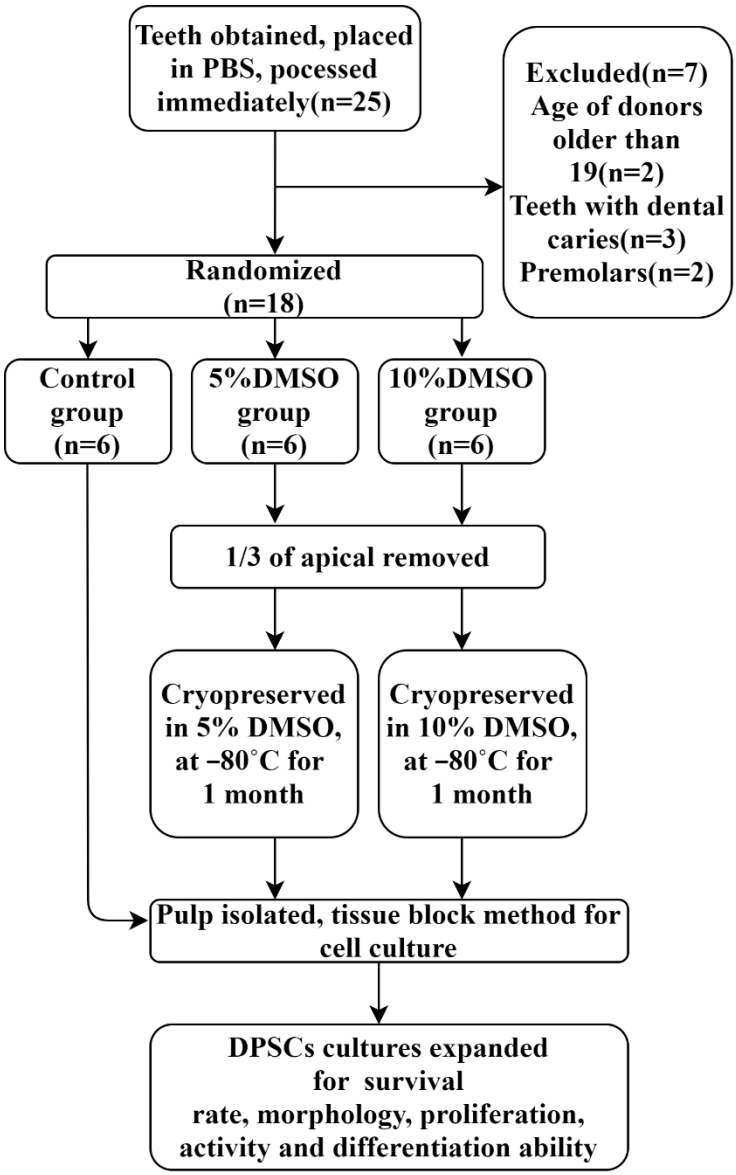



**Figure 1.** Flow diagram for experimental processing. DPSCs of control group were immediately cultured for cellular characterization experiments. After cryopreservation, other groups of DPSCs were cultivated for experiments.

### *2.2. Cryopreservation*

The 18 third molars were randomly divided into three groups: a control group (C), a 5% DMSO group (T1), and a 10% DMSO group (T2). The teeth in group C were

opened with a high-speed turbine and the pulp tissue was removed. The pulp tissue was cut into small pieces with sterilized ophthalmic scissors and placed in 24-well plates for applanation culture. The apical 1/3 of the teeth in groups T1 and T2 were removed with a high-speed turbine and added to a lyophilization solution containing DMSO (Figure 2). They were stored at 4 °C for 30 min, -20 °C for one hour, and -80 °C for one month, respectively. Afterwards, the cell culture of T1, T2 group cohort was performed using the tissue apposition method described above.

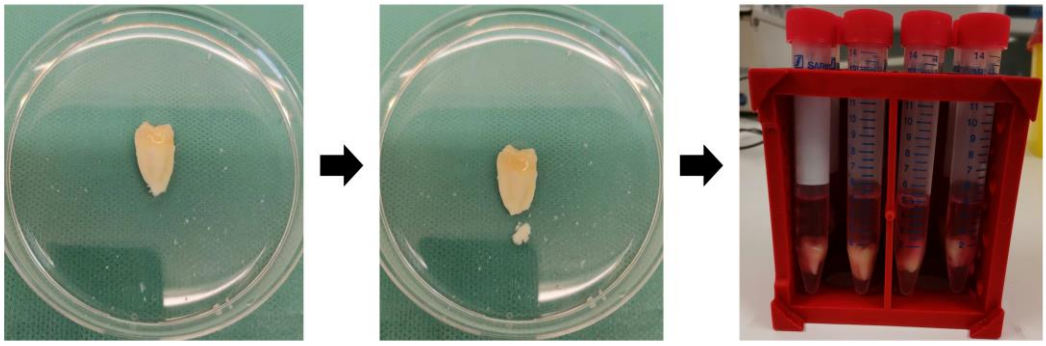


**Figure 2.** The procedure of teeth treatment in T1 and T2 groups.

### *2.3. Culture of Cells*

After the dental pulp tissue blocks were basically adhered to the surface of plates, DMEM cell culture medium was added and placed in a cell culture chamber at 37°C with 5% CO2 for incubation. The culture medium was changed every 3 days, and the cell growth was observed under the microscope every day. After the cell density reached the ideal state, the cells were digested with Trypsin and passaged.

### *2.4. Primary Cellular Morphology and Cell Growth Time*

Every day, dental pulp tissue was inspected with an inverted microscope, and the period from tissue block implantation to cellular adhesion and expansion was recorded in each group.

### *2.5. Flow Cytometry*

hDPSCs were prepared for flow cytometry. PE-conjugated antibodies were selected, including CD34, CD45, CD73, CD90 and CD105, to test the cells following protocol. The number of each sample include $2 \times 10^5$ cells. The concentration of the dye liquid is 1:1000 live/dead dye and 1:100 antibody. hDPSCs were stained and measured with BD LSRFortessa cell analyzer and BD FACSDiva software V6.1.3. Data were then analyzed with FlowJo software.

### *2.6. Colony-Forming Efficiency*

$1 \times 10^3$ hDPSCs per group were inoculated into 10 cm diameter cell culture plates and cultured at 37°C for 2 weeks. After fixing the cells with methanol and performing Giemsa staining, the number of colonies with more than 50 hDPSCs was counted and the colony-forming efficiency was calculated. The calculation formula was as follows: Colony-forming efficiency = number of available colonies / number of seeded cells × 100%

### *2.7. Cell Survival Rate*

The first generation hDPSCs were stained with 0.4% Taipan Blue, and the live cells

not stained with blue were counted microscopically in 500 cells and the cell survival rate was calculated. The calculation formula was as follows: Cell survival rate of Trypan Blue = number of unstained living cells/500 × 100%

Live–dead staining: The $8 \times 10^4$ hDPSCs were inoculated on TCC, incubated for 4 h at 37 °C and stained with fluorescein diacetate and propidium iodide staining solution. The number of green-stained live and dead erythrocytes in the field of view was counted microscopically and the cell survival rate was calculated using the following formula. Cell survival rate of live–dead staining = number of green-stained cells/number of total cells × 100%

### *2.8. Proliferation Testing with MTS Assay*

hDPSCs of third generation in each group were inoculated into 96-well plates at $2 \times 10^3$ cells per well and incubated at 37 °C. After incubation for 3 h per day for 8 consecutive days with the addition of MTS mix according to protocol, readings were measured at 490 nm using a microplate reader.

### *2.9. Differentiation Potential Assessment*

Adipogenic differentiation: hDPSCs of third generation in each group were inoculated into 6-well plates at $4 \times 10^4$ per well and incubated at 37 °C. The cells were induced by adding adipogenic induction solution for 3 weeks after the cells had adhered to the wall and reached 60-70% density. Afterwards, cells were fixed, stained with 0.5% Oil Red O solution and observed for lipid droplet formation.

Adipogenic differentiation quantitative analysis: 1 ml of isopropanol was added to each well to dissolve the stained lipid droplets and the supernatant was transferred in 100 μL per well into a 96-well plate and read at 540 nm.

Osteogenic differentiation: hDPSCs of third generation in each group were inoculated into 6-well plates at $4 \times 10^4$ per well and incubated at 37 °C. The cells were induced by adding adipogenic induction solution for 3 weeks after the cells had adhered to the wall and reached 60-70% density. Afterwards, cells were fixed, stained with 0.1% Alizarin red S solution and observed for calcium nodules formation.

Osteogenic differentiation quantitative analysis: Add 750 μL of 10% acetic acid per well and after 5 minutes add 750 μL of 10% ammonium hydroxide to dissolve calcium nodules and perform the neutralization reaction. The solution was transferred in 100 μL per well into a 96-well plate and read at 405 nm.

### *2.10. Osteogenic Activity with ALP Assay*

According to the instructions of the ALP kit, three groups of wells need to be set up to determine ALP activity: standard wells, sample wells and sample background control wells. First, 120μL of standard solution is added to each of the standard wells, and 80μL of osteogenic induction supernatant is added to the sample wells and sample background control wells. Then 20 μL of stop solution is added to each sample background control well, 50 μL of 5 mM pNPP solution is added to each sample well and sample background control well, and 10 μL of ALP enzyme solution is added to each standard well. After incubation at 25 °C for 60 min, 20 μL of the stop solution

was added to each sample well to stop the reaction. Results were measured at 405 nm using a microplate reader. ALP activity assays were performed at 1, 2 and 3 weeks after the start of induction, respectively.

### *2.11. Gene Expression Detection*

Each group of mRNAs was extracted by TRIzol method at 1, 2 and 3 weeks after the start of induction, respectively, and reverse transcribed into cDNA and subjected to real-time fluorescence quantitative PCR and conventional PCR and gel electrophoresis, respectively. The results of real-time fluorescence quantitative PCR were analyzed using the $2^{-\Delta\Delta Ct}$ method to calculate the expression of relevant mRNAs in each group. The designed primer sequences are shown in Table I.

**Table I.** ***Primer sequences of adipogenic and osteogenic induced gene expression.***

| Primer | Direction | Sequence | Length of products (bp) |
|---|---|---|---|
| LPL | Forward | ACAAGAGAGAACCAGACTCCAA | 76 |
| | Reverse | GCGGACACTGGGTAATGCT | |
| SPPAR-γ | Forward | GGGATCAGCTCCGTGGATCT | 186 |
| | Reverse | TGCACTTTGGTACTCTTGAAGTT | |
| ALP | Forward | ACTGGTACTCAGACAACGAGAT | 97 |
| | Reverse | ACGTCAATGTCCCTGATGTTATG | |
| RUNX 2 | Forward | TGGTTACTGTCATGGCGGGTA | 97 |
| | Reverse | TCTCAGATCGTTGAACCTTGCTA | |
| Type I collagen | Forward | GGACACAATGGATTGCAAGG | 441 |
| | Reverse | AACCACTGCTCCACTCTGG | |
| Osteocalcin | Forward | GGCGCTACCTGTATCAATGG | 110 |
| | Reverse | GTGGTCAGCCAACTCGTCA | |
| GAPDH | Forward | GAGTCAACGGATTTGGTCGT | 185 |
| | Reverse | GACAAGCTTCCCGTTCTCAG | |

### *2.12. Statistical Analysis*

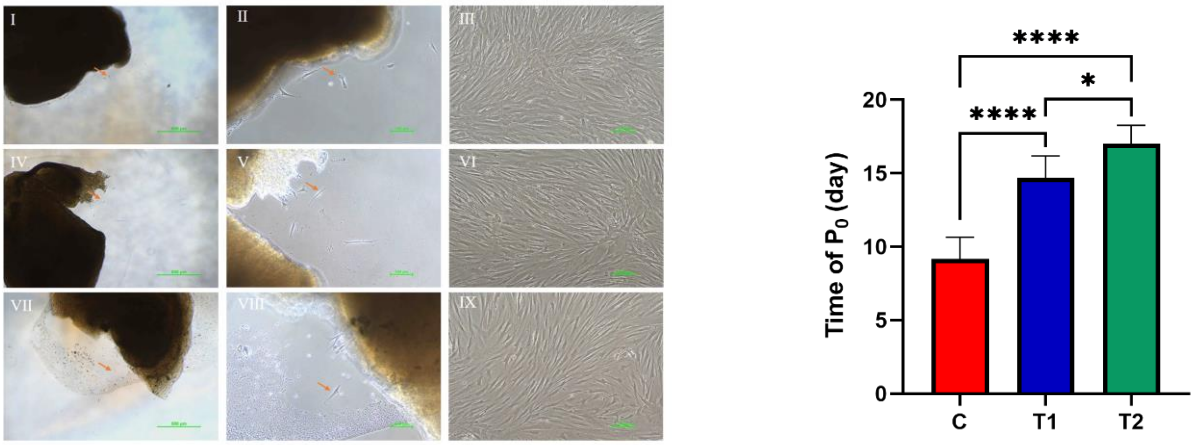
Student *t*-tests were utilized to analyze the means of difference among groups. *p*-

values lower than 0.05 were defined statistically significant. SPSS 25.0 and Graphpad Prism V9.0 were used for analysis and graphing.

# 3. Results

### *3.1. Cell morphology and Minimum Appearance Time*

The growth and minimum appearance time of hDPSCs around tissue blocks were examined daily under light microscopy. After microscopic assessment of cell morphology by two experienced experimenters, the cell morphology of the T1 and T2 groups was essentially identical to that of the C group (κ = 88.9% and 83.3%). The cells were all triangular or spindle-shaped, with deeply stained cytoplasm and well-defined nucleus (Figure 1A). However, the minimum time of appearance of hDPSCs was significantly longer in T1 and T2 groups (16.830 ± 1.472 and 14.670 ± 1.506 days, respectively) than in group C (9.170 ± 1.472 days). The difference was statistically significant (*$p$<0.01).




(**A**) (**B**)

Figure 1. Morphology of hDPSCs in T1, T2 and C groups. (A) Morphology of dental pulp tissue and cells at the primary and the third generations. I–III: C group—Primary

cells emerged from tissue blocks (×40; ×100) and the cells from the third generation after passage (×100). IV–VI: T1 group—Primary cells emerged from tissue blocks (×40; ×100) and the cells from the third generation after passage (×100). VI–IX: T2 group—Primary cells emerged from tissue blocks (×40; ×100) and the cells from the third generation after passage (×100). (B) The first appearance time of dental pulp cells in different groups: The first appearance time of dental pulp cells in the frozen groups was significantly longer than in the C group, and the first appearance time in the T2 frozen group was significantly longer than that in T1 group. The cryopreservation approach led to a longer period for cells to grow out of the tissue blocks. (* $p < 0.05$, **** $p < 0.0001$.)

### *3.2. Primary Cell Yield*

After cell colonies were matured, they were digested and counted separately for each group of hDPSCs. the number of primary hDPSCs collected in groups T1 and T2 ($(6.333 \pm 1.341) \times 10^5$ and $(6.658 \pm 1.229) \times 10^5$) was significantly lower than that in group C ($(9.725 \pm 1.601) \times 10^5$), and the difference was statistically significant (** $p < 0.01$) (Figure 2).

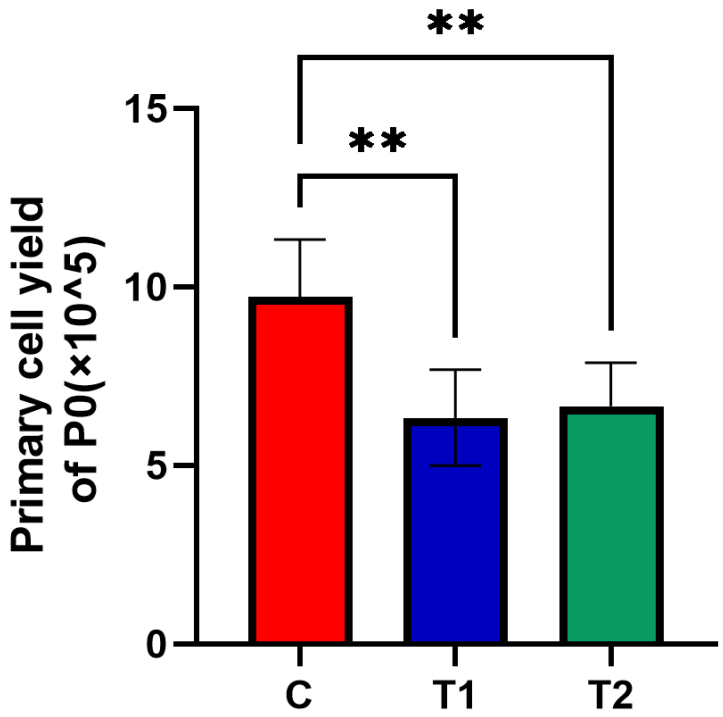



**Figure 2.** Effects of new cryopreservation strategy on the primary cell yield. The new cryopreservation strategy dramatically decreased the number of harvested primary cells compared with the C group (** $p < 0.01$).

### *3.3. Detection of MSCs Specific Antigens*

All three groups of hDPSCs expressed MSCs-specific antigens: CD73, CD90 and CD105 were more abundantly expressed, while CD34 and CD45 were less abundantly expressed, which is consistent with the typical characteristics of MSCs (Figure 3).

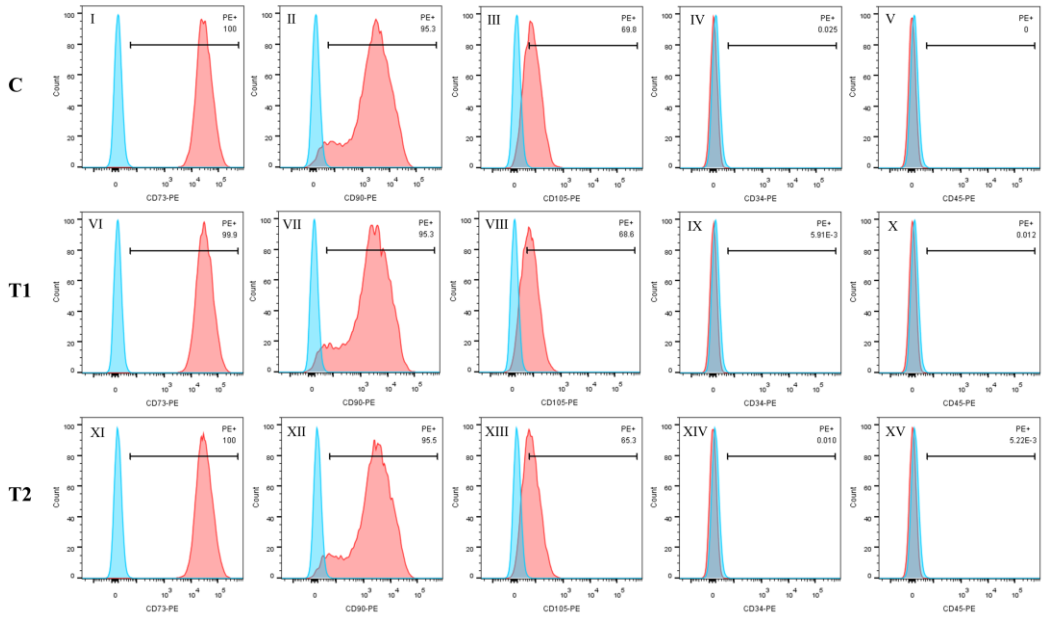
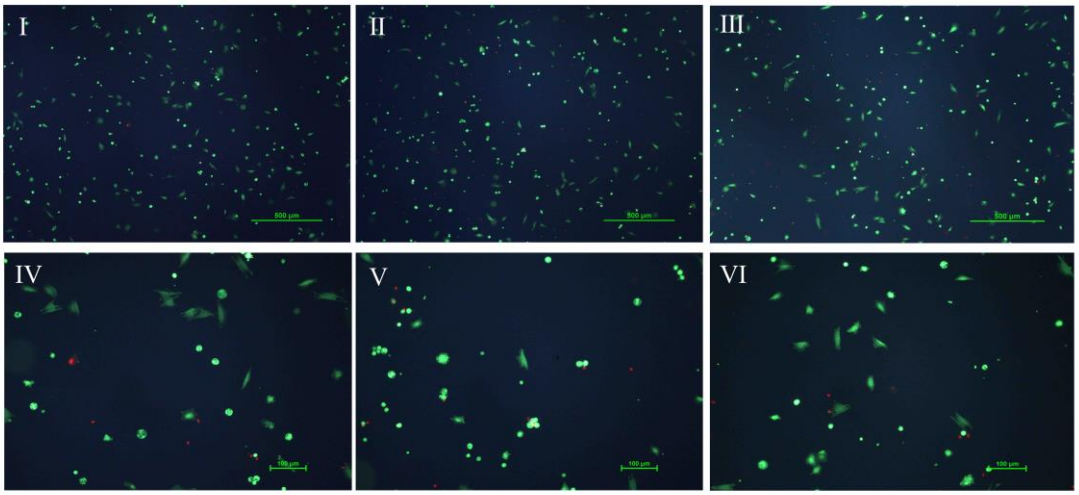


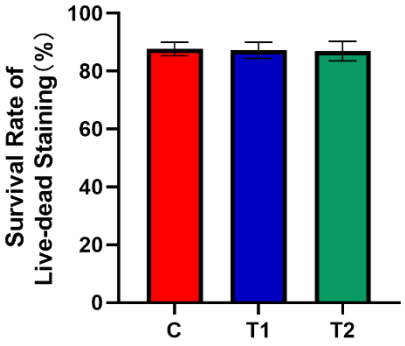

**Figure 3.** Identification of hDPSCs surface markers by flow cytometry. I–V: C group; VI–X: T1 group; XI–XV: T2 group. The expression of CD73, CD90 and CD105 was positive; otherwise, CD45 and CD34 expression was negative in the three groups.

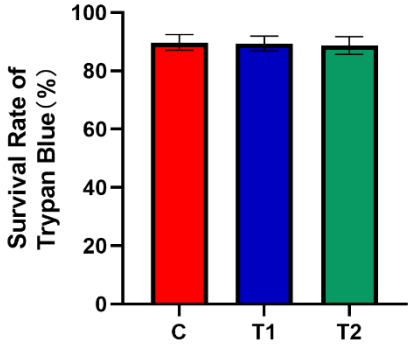
### *3.4. Cell Survival Rate*

Calculations of the surviving hDPSCs after Trypan Blue staining and live-dead staining showed similar proportions of viable cells in the three groups, with no statistically significant differences ($p > 0.05$) (Figures 4B,4C).




(**A**)



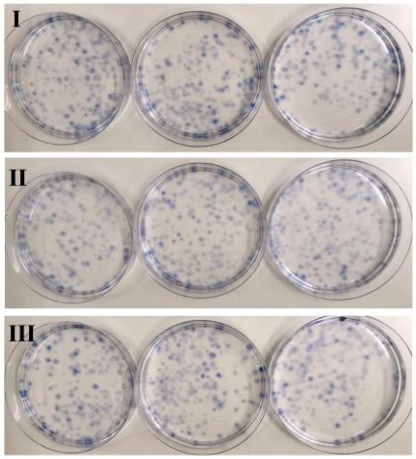

(**B**) (**C**)

**Figure 4.** Effects of new cryopreservation strategy on the cell survival rate. (**A**) Live–dead staining results of hDPSCs. I: C group (×40); II: T1 group (×40); III: T2 group (×40); IV: C group (×100); V: T1 group (×100); VI: T2 group (×100). (**B**) The survival rate of live–dead staining in each group. (**C**) The survival rate of Trypan Blue staining in each group.

### *3.5. Cell Proliferation Abilities*

The cell colony-forming efficiency and cell proliferation curves of hDPSCs were

calculated separately, and the results showed no statistical difference in the clone formation rate and daily absorbance of hDPSCs between the three groups ($p > 0.05$) (Figure 5B,5C).

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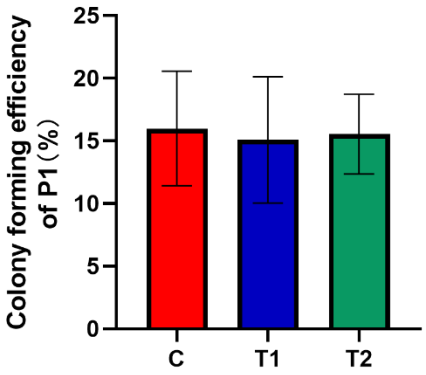


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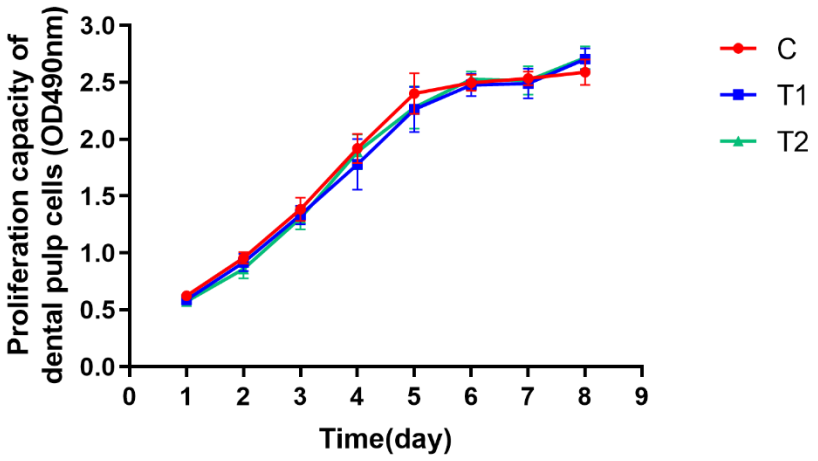
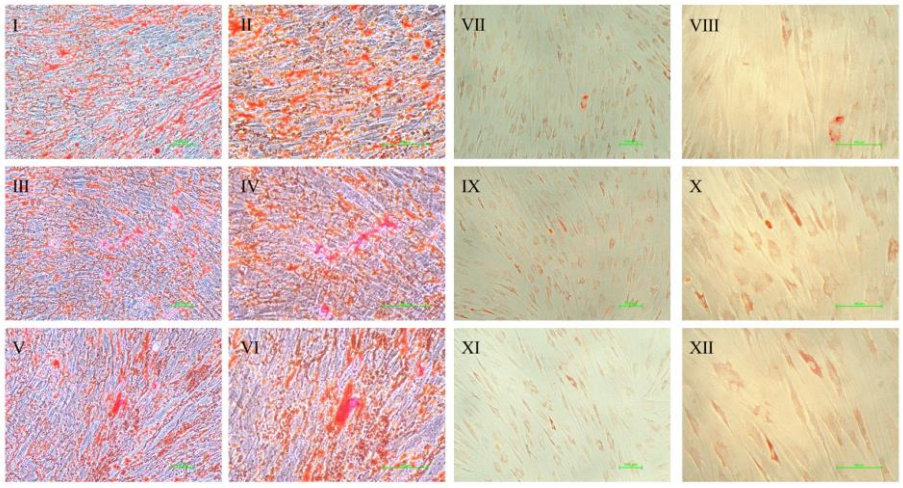



(**C**)

**Figure 5.** Proliferation ability of primary hDPSCs in each group. (**A**) Giemsa staining results of hDPSCs. I: C group; II: T1 group; III: T2 group. (**B**) Colony-forming efficiency. (**C**) Cell growth curve (MTS assay).

### *3.6. Differentiation Potential of hDPSCs*

Alizarin Red and Oil Red O staining was performed on hDPSCs after 3 weeks of osteogenic and lipogenic induction, respectively (Figure. 6A), and the morphology and density of osteochondral nodes and lipid droplets were similar in the three groups as microscopically. The absorbance of the solutions of the three groups was measured after lysis with the corresponding solvents and the results were not statistically different ($p > 0.05$) (Figure 6B, 6C).

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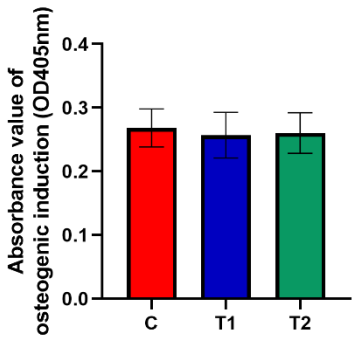


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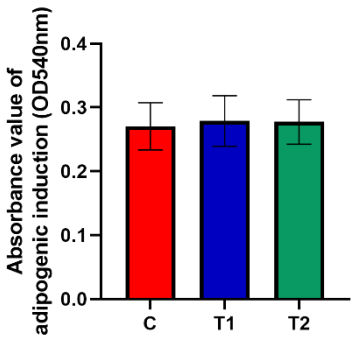


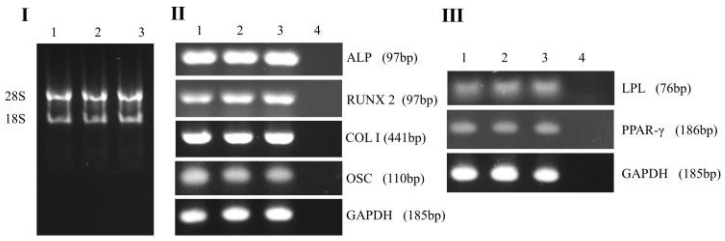

(**C**)

**Figure 6.** Differentiation potential of hDPSCs. (**A**) Osteogenic and adipogenic

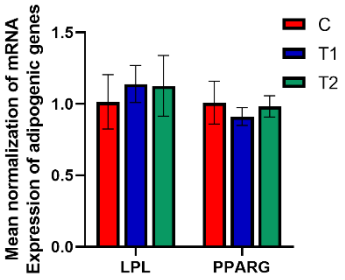
differentiation results of hDPSCs. I–VI: Osteogenic differentiation results. I: C group (×40); II: C group (×100); III: T1 group (×40); IV: T1 group (×100); V: T2 group (×40); VI: T2 group (×100). VII–XII: Adipogenic differentiation results. VII: C group (×40); VIII: C group (×100); IX: T1 group (×40); X: T1 group (×100); XI: T2 group (×40); XII: T2 group (×100). (**B**) The absorbance value at 450 nm of dissolved solution after Alizarin Red S staining in the three groups. (**C**) The absorbance value at 540 nm of dissolved solution after Oil Red O staining in the three groups.

### *3.7. Gene Expression Profile of hDPSCs*

The relative expression of osteogenic and adipogenic genes of hDPSCs in the three groups did not differ statistically significantly at the same time point ($p > 0.05$). However, there were some changes in the relative expression of osteogenic genes at 7, 14 and 21 days (Figure 7A-C).




(**A**)

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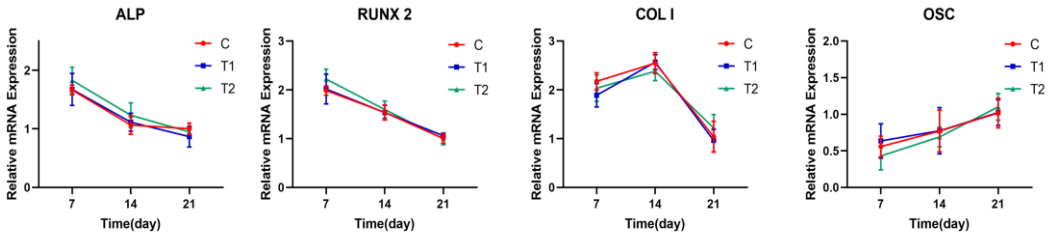


(**C**)

**Figure 7.** Representative gene expression profile in different groups. (**A**) Gel electrophoresis results. I: RNA electrophoresis of three groups. 1: C group; 2: T1 group; 3: T2 group. II: Electrophoresis results of osteogenic expression and reference genes in each group. 1: C group; 2: T1 group; 3: T2 group; 4: Negative control group. III: Electrophoresis results of adipogenic expression and reference genes in each group. 1: C group; 2: T1 group; 3: T2 group; 4: Negative control group. (**B**) Quantitative real-time PCR results of adipogenic genes. (**C**) Quantitative real-time PCR results of osteogenic genes.

### *3.8. ALP Assay Test Results of hDPSCs*

The activity of ALP in the supernatant decreased significantly with increasing time of osteogenic induction. However, there was no significant difference in ALP activity between the three groups of hDPSCs at the same time point ($p > 0.05$, Figure 8).

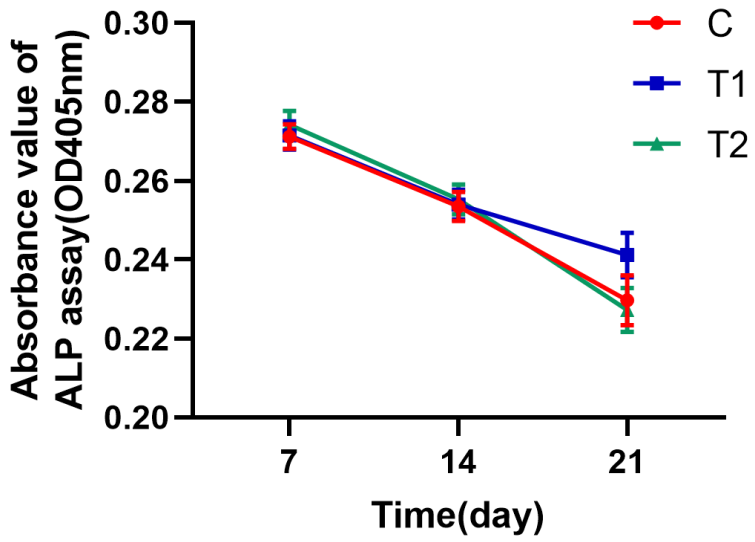


**Figure 8.** ALP assay results of hDPSCs in each group.

## 4. Discussion

In this study, we have used a new, simpler and faster method for the rapid and timely preservation of donor third molars in order to facilitate their future use in cell therapy. Eighteen healthy molars from young participants were selected randomly and subjected to experiments to assess whether the cell biological properties of hDPSCs were affected by the new method of cryopreservation. Overall, the results of the experiment were relatively satisfactory. The first appearance of cells in the T1 and T2 cryopreservation groups took a longer time than in the C group, and the above time was longer in the T2 group than in the T1 group. In addition, significantly more primary hDPSCs were collected in group C than in groups T1 and T2, suggesting that DMSO prolonged the growth time of primary hDPSCs. However, after being passaged, there was no significant difference in the morphology of the first generation

hDPSCs from the three groups, and flow cytometric identification according to the International Society for Cell Therapy's (ISCT) guidelines[9] also showed that all three groups of cells still strongly expressed the characteristic surface markers of MSCs including CD73, CD90 and CD105, while CD45 and CD34 expression was negative, demonstrating that the new freezing method did not affect the stemness of the hDPSCs. Furthermore, the survival rate of first-generation hDPSCs in the three groups was no longer different, and their daily proliferation curve readings were essentially the same, indicating that the cells in the T1 and T2 freezing groups were in a normal growth state. More importantly, the gene expression related to osteogenic and adipogenic differentiation outcome of hDPSCs in the third-generation cryopreservation group was not statistically different from that in group C, illustrating that the new cryopreservation method does not adversely affect the differentiation ability of hDPSCs.

The present study demonstrates that the new method of teeth cryopreservation may adversely affect the harvest and emergence time of primary hDPSCs, probably due to the small contact area between the cryopreservation solution and the pulp tissue, and that some hDPSCs are not sufficiently protected during the freezing process. However, after passaging, the cell activity and biological properties of the hDPSCs in the freezing group remained essentially normal, indicating that one month of freezing at -80 °C does not reduce cell performance. On the other hand, this may also be related to the younger age of the tooth donors and the greater tolerance of hDPSCs to physical and chemical stimulation.

Several studies have been carried out to investigate the effect of freezing hDPSCs under various conditions. Pilbauerova N et al. stored hDPSCs frozen in 10% DMSO at -80 °C for one year and found that hDPSCs maintained their original stemness, viability and ability to proliferate and differentiate[10]. Shalini R et al. even resuscitated hDPSCs after freezing them at -80 °C for up to 5 years and found that the thawed hDPSCs maintained a stable stem cell function and immunophenotype[11]. Similarly, Erik J W et al. showed that hDPSCs stored at -85 °C for six months still do not lose their biological properties[12]. Ginani F et al. examined deciduous tooth-derived stem cells after six months of freezing in 10% DMSO and determined that the cells had a similar proliferative capacity and cell cycle as before freezing[13]. In addition, in preliminary experiments in our laboratory where dental pulp tissue was cut up and frozen in 5% and 10% DMSO for 1 month, the ability of hDPSCs to proliferate and differentiate remained unaffected[14]. These experiments and the results obtained in the present experiment are similar and demonstrate that the new freezing and storage method has a limited effect on the biological properties of hDPSCs. Due to their high proliferative capacity and multi-directional differentiation potential, including differentiation into osteoblasts, lipoblasts, chondrocytes, neuronal cells and islet-like cells[15,16], hDPSCs are considered ideal candidates for tissue engineering and have been extensively studied. Moreover, hDPSCs have significant advantages over other sources of stem cells, namely easy access to tissue, less damage to the donor site and less expense; as common sources of hDPSCs usually include third molars[17], multiple teeth[18] and teeth that need to be extracted for orthodontic purposes[19], which are often

considered discarded tissue. Nevertheless, as the longer an isolated tooth is exposed in vitro, the smaller quantity of hDPSCs that can be isolated[20], and the prolonged exposure to the in vitro environment further increases the risk of dental tissue being infected. Our study demonstrates the feasibility of a new method of preserving intact healthy teeth directly at -80°C, which is capable of preserving hDPSCs to maintain optimal activity while minimizing in vitro exposure time and the potential for infection. More importantly, the new preservation method significantly reduces the procedures and requirements needed to preserve hDPSCs, effectively setting the stage for the establishment of dental stem cell banks and tissue engineering applications in the future.

## 5. Summary

Our study used 5% and 10% concentrations of DMSO to cryopreserve healthy third molars at -80 °C to observe whether the new cryopreservation method had an effect on hDPSCs. The experimental results showed that the new cryopreservation strategy had an effect on the growth and culture of primary hDPSCs, decreasing the number of cells harvested while increasing the time of appearance for hDPSCs. However, after being passaged, the biological properties of hDPSCs were barely affected, maintaining a proliferation and differentiation capacity consistent with that of unfrozen hDPSCs, with no statistically significant differences ($p>0.05$). The new cryopreservation method ensures that the cells are in optimal condition while reducing the time that the isolated teeth are exposed to the in vitro environment, thereby

reducing the likelihood of tissue infection. Furthermore, the new strategy reduced the steps and conditions for freezing and storage, thus allowing for a significant further reduction in the cost of preserving hDPSCs, which could provide a theoretical basis for the establishment of dental stem cell banks and further applications of hDPSCs in tissue engineering.

# 6. Zusammenfassung

In unserer Studie wurden gesunde dritte Molaren mit 5 % und 10 % DMSO-Konzentrationen bei -80 °C kryokonserviert, um festzustellen, ob die neue Kryokonservierungsmethode Auswirkungen auf hDPSCs hat. Die experimentellen Ergebnisse zeigten, dass sich die neue Kryokonservierungsstrategie auf das Wachstum und die Kultur von primären hDPSCs auswirkte, indem sie die Anzahl der geernteten Zellen verringerte und gleichzeitig die Zeit des Auftretens von hDPSCs verlängerte. Nach der Passage wurden die biologischen Eigenschaften der hDPSCs jedoch kaum beeinträchtigt. Sie behielten eine Proliferations- und Differenzierungskapazität bei, die mit der von ungefrorenen hDPSCs übereinstimmte, wobei es keine statistisch signifikanten Unterschiede gab ($p > 0.05$). Die neue Kryokonservierungsmethode stellt sicher, dass sich die Zellen in einem optimalen Zustand befinden, und verkürzt gleichzeitig die Zeit, in der die isolierten Zähne der In-vitro-Umgebung ausgesetzt sind, wodurch die Wahrscheinlichkeit einer Gewebeinfektion verringert wird. Darüber

hinaus konnten durch die neue Strategie die Schritte und Bedingungen für das Einfrieren und die Lagerung reduziert werden, was eine weitere deutliche Senkung der Kosten für die Konservierung von hDPSCs ermöglicht, was eine theoretische Grundlage für die Einrichtung von zahnmedizinischen Stammzellbanken und weitere Anwendungen von hDPSCs im Tissue Engineering darstellen könnte.

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# III. Authors' contribution

- Wang Wang: Conceptualization, data curation, original draft preparation, review and editing;
- Ming Yan: Conceptualization, original draft preparation, review and editing, funding acquisition;
- Ghazal Aarabi: Software;
- Ulrike Peters: Validation;

- Marcus Freytag: Formal analysis;
- Martin Gosau: Resources;
- Ralf Smeets: Investigation and funding acquisition;
- Thomas Beikler: Methodology, supervision and project administration.

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your pride and gave me a high degree of recognition and sufficient financial support to study abroad in order to complete my studies.

# V. Curriculum vitae

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**Professional Expericence**

1. Since 11. 2019 Department of Periodontics, Preventive and Restorative Dentistry, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
2. 09. 2015- 06. 2018 Southwest Medical University, Luzhou, China
   **Master** in Endodontics
3. 09. 2009- 06. 2014 Southwest Medical University, Luzhou, China
   **Bachelor** in Stomatology

**Experimental skills**

1. Cell culture, flow-cytometry, florescence staining, qPCR, Western Blotting, DNA/RNA and protein extraction and purification, the cultivation of and biochemical identification of bacteria.
2. Animal techniques: animal model with rats and mice, the use of electron microscope and fluorescence microscope, Meta-analysis, Bioinformatics.
3. The use of statistical analysis software such as SPSS, R, Graphpad Prism, Review Manager，Comprehensive Meta Analysis.

**Publications**

1. **Wang W**, Yan M, Aarabi G, et al. Cultivation of Cryopreserved Human Dental Pulp Stem Cells - A New Approach to Maintaining Dental Pulp Tissue[J]. International Journal of Molecular Sciences, 2022, 23(19): 11485.
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Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

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