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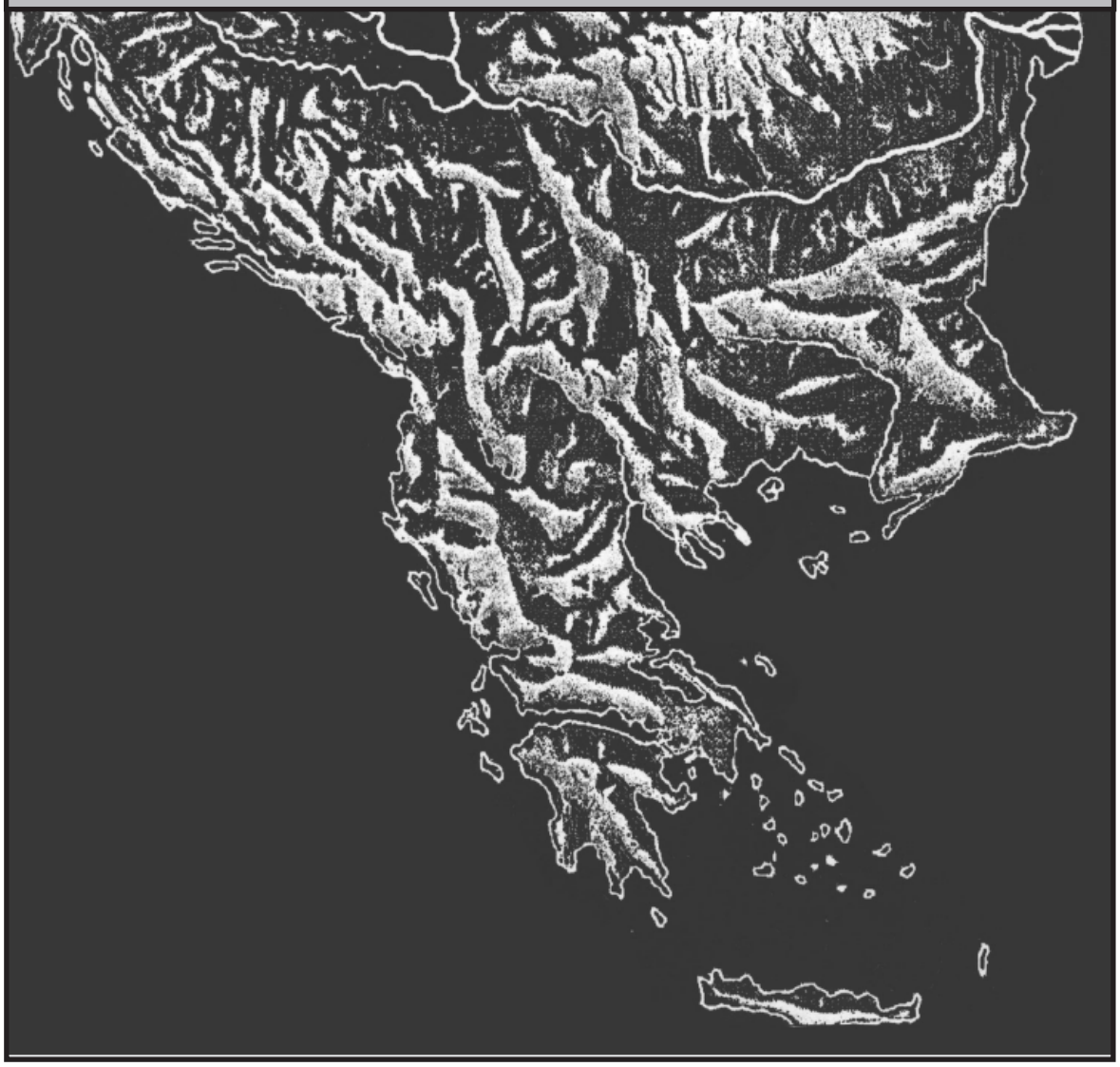

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SUMMARY

Dental pulp stem cells (DPSCs) as postnatal stem cells have recently been described. They are clonogenic cells, capable for self-renewal with high proliferative potential. Their multilineage potential and plasticity enables their differentiation into different kind of cells, such as osteoblasts, chondrocytes, adipocytes, muscle cells, neural cells, odontoblasts, cementoblasts and ameloblasts. DPSCs are an important human stem cells source, especially in patients who lost their chance for umbilical cord blood isolation and preservation. As these cells became useful for tissue engineering and cell therapy, proper mode of their preservation also became important. The most important points in the cryopreservation and recovery procedure are: growth phase of harvested cells, number of cells, the proper cryopreservative concentration and serum concentration. The cryopreservation process includes the following general components: harvesting of the cells, addition of cryopreservative, the freezing procedure, the thawing procedure and assessment of the viability prior to transplantation. There is no single and perfect cryopreservation method. Further investigations should be regarding capability of DPSCs and their differentiated cells to recover and restart proliferation, differentiation and new tissue production for therapeutic use after cryopreservation.

Keywords: Dental Pulp; Stem Cells; Cryopreservation

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Postnatal Stem Cells and Their Potential Use in Dentistry

Postnatal stem cells are defined as clonogenic cells capable for self-renewal and multilinear differentiation[1,2]. They play a significant role in tissue repair and regeneration[3]. Advances in stem cell biology and gene therapy technology have provided the great potential of postnatal stem cells for use in regenerative dentistry[4-6]. Recent investigations identified and characterized stem cells from dental tissues, such as: dental epithelium, dental papilla, dental pulp, periodontal ligament and dental follicle[3,4,7,8]. Dental pulp stem cells have recently been described as similar to bone marrow stem cells regarding their gene expression profiles and expression of cell markers[9,10]. Even though dental pulp stem cells have not been as widely studied as bone marrow stem cells, their high proliferative potential, self-renewal and multilinear differentiation potential has been described. DPSCs possess multilineage potential and plasticity, so they are able to differentiate into different kind of cells, such as osteoblasts, chondrocytes, adipocytes, muscle cells, neural cells, odontoblasts, cementoblasts and ameloblasts[11].

A growing number of niches containing significant number of stem cells have been identified within the human body, which represents a physiologic source of stem cells suitable for regeneration, transplantation and tissue-based therapies. Dental tissues, such as adult dental pulp, periodontal ligament and dental pulp from exfoliated deciduous teeth, are shown to be an approachable and large source of stem cells[12]. Stromal bone producing dental pulp stem cells (SBP-DPSCs) and stromal bone producing-stem cells from human exfoliated deciduous teeth (SBP-SHEDs) can produce tridimensional bone in *in vitro* conditions. This bone is woven but, after *in vivo* transplantation, it can be remodelled into lamelar bone[8,13].

Dental Pulp Stem Cells

Dentine-pulp complex possess natural reparative and regenerative potential which enables formation of tertiary dentine[3]. After mild stimuli, such as attrition, erosion or superficial caries, odontoblasts secrete reactionary dentine matrix. However, intensive stimuli, such as trauma, advanced caries or extensive restaurative procedures, may cause death of odontoblasts and a cascade of specific signals and yet un-clarified events. This leads to high proliferation rate of progenitor/stem cells and their differentiation into new odontoblasts[3,14-16]. New odontoblasts secrete atubular reparative dentin or osteodentin[17]. This reparative dentine represents a layer of mineralised tissue that provides protection of pulp tissue vitality[3].

Postnatal pulp comprises several niches with potential progenitor/stem cells which may have a significant role in formation of reparatory dentine. This subpopulation of undifferentiated cells represents approximately 1% of total cell count, and it is inactive in healthy tissue environment. Although very small, this subpopulation has extremely high proliferative potential and multi/potential capacity to produce terminally differentiated cells in response to specific extracellular signals[3,18].

DPSCs are important human stem cells source, especially in patients who lost their chance to isolate and preserve stem cells using umbilical cord blood samples, as this is one-time-only opportunity to obtain the specimen, at the time of birth[18]. However, dental banking offers convenience to harvest specimens during the period of mixed dentition. An important issue in DPSCs banking is limited in volume of dental pulp tissue within the tooth cavity. Therefore, possible progenitor/stem cells expansion after adequate method of preservation is very important as this may augment potential therapeutic use of DPSCs in regenerative medicine and dentistry[19].

Stem Cell Preservation

As progenitor/stem cells became useful for tissue engineering and cell therapy applications, proper mode of their preservation also became important. Stem cells may be used immediately after isolation in only certain situations when allogenic transplantation procedures are applied. It is defined that progenitor/stem cell transfer from donor to recipient should be established within 72 hours. Therefore, in some cases, protocols for preliminary storage on supra-freezing temperatures can be used[20]. A long-term preservation til potential therapeutic use demands cryopreservation of progenitor/stem cells. This method is proven to be safe without significant adverse outcomes regarding host reaction, graft failure and transplantation success.

The donor specimens are usually stored in public or private banks, which store donor specimens for the donor himself (private banks), or for an unknown matching recipient during indeterminate time period. Public banks are non-profit organizations that provide donor specimens to match recipients through national or international bank registries. Haematopoietic stem cells from umbilical cord blood have been extensively studied regarded to possible recovery and therapeutic use in stem cell transplantation procedures after cryopreservation[21]. Current data have shown that there are about 170 000 frozen units in 37 cord blood registries in 21 countries world wide[20].

Cryopreservation Methods

There is no single and perfect cryopreservation method. During the past decade, different transplantation centres developed their individual techniques for long-term preservation of progenitor/stem cells and their differentiated cells[20,22-23]. The identification, isolation and characterization of DPSCs, as an approachable human postnatal stem cells source, enabled their exploitation[24,25]. Therefore transplantation centres adjusted their methods for long-term preservation of DPSCs as they became an approachable source of human stem cells for auto-transplantation, tissue regeneration and tissue-based clinical therapies in humans[9-10,13,26]. Cryopreservation and recovery procedures are described and debated in the literature and the most important points in the procedure are: growth phase of harvested cells, number of cells (too low or too high cell number may decrease the recovery rate), the proper cryopreservative concentration and serum concentration[18,20]. The cryopreservation process includes the following general components:

- Harvesting of the donor cells;
- Addition of the proper cryopreservatives;
- The freezing procedure at the proper temperature and freezing rate;
- The thawing procedure and
- Assessment of the viability of the specimen prior to transplantation procedure.

Cryopreservation procedures undertake international recommendations and rules. The International Society for Cellular Therapy (ISCT) defined special gamma irradiated, ethinyl vinyl acetate based cryostorage container products in which the specimens should be preserved. It has been shown that the use of different containers, PVC and polyolefin plastic bags accomplished different results regarding the viability of specimens[20].

Cryopreservative is additive to stem cell concentrate, which intercepts formation of intracellular and extracellular crystals and consequent cell death. The standard cryopreservative is dimethyl sulfoxide (DMSO), which prevents cell lesions during freezing. It is usually used at concentrations of 1-20%, but common

concentration is 10% in combination with saline and serum albumin[13,18,20,23,26-27]. *In vitro* study proved that 10% DMSO was superior to lower concentrations[28]. However, proper DMSO concentration is debated in the current literature due to DMSO induced toxicity with significant side effects after transplantation of stem cells. Side effects, such as nausea, vomiting, cardiovascular, respiratory, CNS, hepatotoxic and haemolytic manifestations were reported[20]. Potential alternative preservation methods with possible future perspective could be propylene-glycol, combination of alpha tocopherol, catalase, ascorbic acid and the glucose dimer trehalose, and addition of the caspase inhibitor zVAD-fmk[20,29]. However, the composition of the cryoprotective medium may be adapted to the respective tissue to be frozen and thus the method of preservation can be further adjusted[23].

The storage temperatures used for cryopreservation of haematopoietic stem cells during the last decade have been -196ºC for storage in liquid nitrogen, -156ºC for storage in vapour nitrogen and -80ºC for storage in cryopreservation mechanical freezers[20,23]. Few authors described possible storage at supra-freezing temperature, at +4ºC[30]. Standard DPSCs preservation procedures described in the literature imply storage in liquid nitrogen at -196ºC and -80ºC[9-10,13,20] or in mechanical freezers at -80ºC[18]. The cells are cooled in a controlled manner and it is possible to choose the point in time of intracellular ice formation at a temperature of approximately -7 to -12°C, preferably -10°C. In this way, the cells' exposure to stress during the freezing process is reduced, and the number of viable stem cells is further increased. After the ice formation, the DPSCs can be cooled down in cooling rates to a temperature between -90°C and -160°C, or between -100°C and -150°C, or even between -120°C and -130°C if permanently storaged[23].

Controlled thawing procedure considers gradual thawing and slow rates, which enables good after thawing recovery[18,27]. Regarding the survival rate of the cryopreserved DPSCs, it has been found important if the cells are thawed in several steps by dilution of the freezing medium[23]. The standard thawing technique is warming in a liquid medium at 35-39ºC, followed by washing or dilution, which reduces the cryopreservative content[13,18,20,23]. The dilution includes replacement of freezing medium with a medium containing 50%, 25%, 12.5%, 6.25% and 0% foetal calf serum[23].

Microbial contamination of transplants represents a significant hazard; therefore, infection must be eliminated before the beginning of cryopreservation method. There is the risk of contamination of the culture bottles, which is not induced by the donor graft, and therefore specific measures should be employed. Also, infected donor specimen must be aborted. In order to prevent contamination, there is a need for processing in absolutely clean areas with monitoring of stem cell preservation procedures and screening of donor specimens.

Papaccio et al[13] have proved that DPSCs and their differentiated cells can be safely recovered after long-term cryopreservation, prolonged for up to 2 years. However, cryopreservation of the whole dental pulp tissue has proven to be problematic. Papaccio et al[13] obtained negative results for the cryopreservation of the whole dental pulp using a similar technique as Seo et al[26] for minced periodontal ligament. This may be the consequence of higher water content in the dental pulp, which is a mucus connective tissue[13]. Therefore, a different cryopreservation procedure is necessary for the whole pulp.

Conclusions

While cryopreservation of isolated and expanded DPSCs has proven to be a safe procedure, storage of primary tissue has shown small recovery rate[10,13,26]. Investigation of different cryopreservation methods could provide evidence about ideal and uniform technique for long-term preservation. Ideal cryopreservation method should provide storage of whole primary tissue and possible isolation, recovery, viability and functionality of DPSCs after thawing, compared to cells isolated from fresh tissue. This idea has the practical and reasonable advantages, because cryopreservation of the tissue specimens in the clinic where they were harvested would be less costly and more effective than direct isolation and preservation of stem cells, which may require additional equipment and professional personnel. There is necessary for further investigations regarding capability of DPSCs and their differentiated cells to recover and restart proliferation, differentiation and new tissue production for therapeutic use after cryopreservation.

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References

1. *Gronthos S, Brahim J, Li W, Fisher L, Cherman N, Boyde A, et al*. Stem cell properties of human dental pulp stem cells. *J Dent Res*, 2002; 81:531-535.
2. *Bianco P, Riminucci M, Gronthos S, Robey P*. Bone marrow stromal stem cells: Nature, biology, and potential applications. *Stem Cells*, 2001; 19:180-192.
3. *Sloan A, Waddington R*. Dental pulp stem cells: What, where, how? *Int J Paediatr Dent*, 2009; 19:61-70.
4. *Todorović V, Marković D, Milošević-Jovčić N, Petakov M, Balint B, Čolić M, et al*. Dental pulp stem cells - potential significance in regenerative medicine. *Stom Glas S*, 2008; 55:170-179.

5. *Maria O, Khosravi R, Mezey E, Tran S*. Cells from bone marrow that evolve into oral tissues and their clinical applications. *Oral Dis*, 2007; 13:11-16.
6. *Ueda M, Yamada Y, Ozawa R, Okazaki Y*. Clinical case reports of injectable tissue-engineered bone for alveolar augmentation with simultaneous implant placement. *Int J Periodontics Restorative Dent*, 2005; 25:129-137.
7. *Ivanovski S, Gronthos S, Shi S, Bartold P*. Stem cells in the periodontal ligament. *Oral Dis*, 2006; 12:358-363.
8. *Laino G, Graziano A, d'Aquino R, Pirozzi G, Lanza V, Valiante S, et al*. An approachable human adult stem cell source for hard-tissue engineering. *J Cell Physiol*, 2006; 206:693-701.
9. *Woods E, Byers M, Calloway E, Bob T*. Cryopreservation of dental pulp for recovery of stem cell populations - a preliminary study. *Cryobiology*, 2006; 53:367-446.
10. *Woods E, Byers M, Hockema J*. Cryopreservation of dental pulp derived stem cell populations: Isolated cells vs. Primary tissue. *Cryobiology*, 2007; 55:324-378.
11. *Mantesso A, Sharpe P*. Dental stem cells for tooth regeneration and repair. *Expert Opin Biol Ther*, 2009; 9(9):1143-1154.
12. *Peng L, Ye L, Zhou X*. Mesenchymal stem cells and tooth engineering. *Int J Oral Sci*, 2009; 1(1):6-12.
13. *Papaccio G, Graziano A, d'Aquino R, Graziano M, Pirozzi G, Menditti D, et al*. Long-term cryopreservation of dental pulp stem cells (sbp-dpscs) and their differentiated osteoblasts: A cell source for tissue repair. *J Cell Physiol*, 2006; 208:319-325.
14. *Téclès O, Laurent P, Zygouritsas S, Burger A, Camps J, Dejou J, et al*. Activation of human dental pulp progenitor/ stem cells in response to odontoblast injury. *Arch Oral Biol*, 2005; 50:103-108.
15. *Smith P, Tchernov E*. Structure, function and evolution of teeth. London and Tel Aviv: Freund Publishing House Ltd, 1992.
16. *Larmas M*. Pre-odontoblasts, odontoblasts, or "Odontocytes". *J Dent Res*, 2008; 87:198; author reply 99.
17. *Lu Y, Xie Y, Zhang S, Dusevich V, Bonewald L, Feng J*. Dmp1-targeted cre expression in odontoblasts and osteocytes. *J Dent Res*, 2007; 86:320-325.
18. *Freshney RI, Stacey GN, Auerbach JM*. Culture of human stem cells. New Jersey: John Willey & Sons, 2007.
19. *Woods E, Perry B, Hockema J, Larson L, Zhou D, Goebel S*. Optimized cryopreservation method for human dental pulp-derived stem cells and their tissues of origin for banking and clinical use. *Cryobiology*, 2009; 59(2):150-157.
20. *Berz D, McCormack E, Winer E, Colvin G, Quesenberry P*. Cryopreservation of hematopoietic stem cells. *Am J Hematol*, 2007; 82:463-472.
21. *Son JH, Heo YJ, Park MY, Kim HH, Lee KS*. Optimization of cryopreservation condition for hematopoietic stem cells from umbilical cord blood. *Cryobiology*, 2010 [Epub ahead of print].
22. *Koliakos G, Alamdari D, Tsagias N, Kouzi-Koliakos K, Michaloudi E, Karagiannis V*. A novel high-yield volume-reduction method for the cryopreservation of uc blood units. *Cytotherapy*, 2007; 9:654-659.
23. *Thie M, Degistirici O*. Method for isolating stem cells from cryopreserved dental tissue. 2009. Available at: http://www.faqs.org/patents/app/20090022693.
24. *Huang J, Gronthos S, Shi S*. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res*, 2009; 88:792-806.
25. *Waddington R, Youde S, Lee C, Sloan A*. Isolation of distinct progenitor stem cell population from dental pulp. *Cells Tissues Organs*, 2009; 189:268-274.
26. *Seo B, Miura M, Sonoyama W, Coppe C, Stanyon R, Shi S*. Recovery of stem cells from cryopreserved periodontal ligament. *J Dent Res*, 2005; 84:907-912.
27. *Moezzi L, Pourfathollah A, Alimoghaddam K, Soleimani M, Ardjmand A*. The effect of cryopreservation on clonogenic capacity and in vitro expansion potential of umbilical cord blood progenitor cells. *Transplant Proc*, 2005; 37:4500-4503.
28. *Balint B, Ivanović Z, Petakov M, Taseski J, Jovcić G, Stojanović N, et al*. The cryopreservation protocol optimal for progenitor recovery is not optimal for preservation of marrow repopulating ability. *Bone Marrow Transplant*, 1999; 23:613-619.
29. *Stroh C, Cassens U, Samraj AK, Sibrowski W, Schulze-Osthoff K, Los M*. The role of caspases in cryoinjury: Caspase inhibition strongly improves the recovery of cryopreserved hematopoietic and other cells. *FASEB J*, 2002; 16:1651-1653.
30. *Ruiz-Argüelles G, Ruiz-Argüelles A, Pérez-Romano B, Marín-López A, Larregina-Díez A, Apreza-Molina M*. Filgrastim-mobilized peripheral-blood stem cells can be stored at 4 degrees and used in autografts to rescue high-dose chemotherapy. *Am J Hematol*, 1995; 48:100-103.

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