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Dental Pulp Stem Cells in Regenerative Therapy

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Abstract

Stem cells, also known as progenitor/precursor cells, have the unique trait of self-renewal and multi-lineage differentiation. Dental stem cells (DSCs) are holding a pivotal role during recent times as they thrive as the cornerstone for the development of cell transplantation therapies that correct periodontal disorders and damaged dentin. DSCs are used therapeutically for different organ systems and numerous diseases, including neurological disorders, diabetes, liver disease, bone tissue engineering, and dentistry. In dentistry, the focus is on predominantly regenerating the pulp and damaged dentin, repairing perforations, and periodontal regenerations. Above all, whole tooth regeneration has been constantly under research. The next decade could be a crucial junction where huge leaps in stem cell-based regenerative therapies could become a reality with successful tissue engineering therapies this could be a biological alternative to synthetic materials that are in use currently. But dental stem cells have their share of challenges for which the research must happen effectively adhering to social responsibilities at all levels.

***Keywords**: Stem cells, Regeneration, Regenerative therapy, SHED.*

Introduction

The human teeth are composed of specific tissues, including the outer mineralized enamel, the dentinal layer adjacent to it, the dental pulp consisting of blood vessels, mesenchymal tissue, and nerves, and the root structure containing the dentin, cementum, and periodontal ligament (PDL) that assist in anchoring the teeth to the alveolar bone [1]. Every organ in the human body originates from the human germ, and the tooth is no different. It's the number of tooth germs formed during the embryonic stage that determines the total number of teeth in an individual [2]. Human tooth formation is the result of the integration of spatiotemporal cellular responses, including proliferation, apoptosis, differentiation, movement, and polarization. Hence, by monitoring the process of cytodifferentiation and organogenesis, there are umpteen possibilities for regenerating specific tissues and replacing specific teeth [2].

The dental pulp has different functions, including protecting the teeth from infections using immunological surveillance, preserving the flexible strength of the teeth to avoid tooth-related fractures, and accelerating dentin formation to avoid external stimuli [3]. Oral health exists right down our priority list despite the worldwide presence of dental caries among a major population of individuals. The problem could be simple, but when the caries is found deep under, dentists prefer to do a pulpectomy [4]. Pulpectomy is the complete removal of the dental pulp without any remnants of the dental pulp stem cells (DPSCs) for regeneration purposes later [5]. But the latest strategy of local

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regeneration therapy of the dentin-pulp complex helps in skipping the pulpectomy process altogether [5]. Stem cells, also known as progenitor/precursor cells, have the unique trait of self-renewal and multi-lineage differentiation. [6]. The ability of stem cells to offer therapeutic possibilities for restoring any structural defects has been the latest buzz among researchers and the dental pulp regenerative capacity of stem cells is evolving well with constant research [6]. Most organs of the human body, including the brain, liver, skin, and blood tissue stem or progenitor cells have restrained differentiation abilities. The dental tissue stem or progenitor cells present in adult tissues help in the repair of the dentin and periodontal ligament [2]. Dental stem cells are holding a pivotal role during recent times as they thrive as the cornerstone for the development of cell transplantation therapies that correct periodontal disorders and damaged dentin. This review article discusses about the application of dental pulp stem cells in Regenerative therapy.

Methodology

The most recognized electronic databases were searched for the data pertaining to the papers that have reported on Regenerative therapy by dental pulp stem cells. PubMed, Google Scholar, Web of Science, Scopus were the keydata bases. Search was carried out for articles published in peer reviewed journals.

Electronic databases searched articles using many Medical Subheadings (MeSH keywords). Regenerative therapy, Dental pulp stem cells, Tooth regeneration, Applications, Regeneration, Endodontic therapy, periodontal regeneration. These key terms were combined in the advanced search using the Boolean operators AND/OR. English language filters were also used. After a thorough search, articles were categorized for their applications.

Applications

Regenerative therapy is the process of replacing/regenerating human cells, organs, and tissues for therapeutic applications [7]. Surprisingly, the concept of regeneration has existed for a long time but wasn't well received until 1932 when the stomatologist G.L. Feldman regenerated dental pulp [6]. But the ground-breaking discovery that altered the course of regenerative therapy didn't happen until the year 2000 when Gronthos et al. isolated and identified an odontogenic progenitor population in the adult dental pulp, and such cells were called dental pulp stem cells (DPSCs) [8]. Dental Stem Cells (DSCs) in humans could be categorized as DPSCs, stem cells from human exfoliated deciduous teeth (SHEDs), stem cells from apical papilla (SCAP), and periodontal ligament stem cells (PDLSCs). DSCs are used therapeutically for different organ systems and numerous diseases, including neurological disorders, diabetes, liver disease, bone tissue engineering, and dentistry. Presently, the focus is on predominantly regenerating the pulp, damaged dentin, repair perforations, periodontal regenerations, and resorbed root, besides using DSCs for quicker healing of the wounds and ulcers, generating salivary proteins and oral microbial colonization [6]. Above all, whole tooth regeneration has been constantly under research.

Regenerative Endodontic Therapy

Any endodontically treated teeth are viable for weakness as a result of dental caries and prosthodontic treatment, thereby increasing the risk of root fracture and even extraction of the tooth [9, 10]. Hence, the purpose of endodontic therapy includes total dentin/pulp complex regeneration that, in turn includes vascularization, pulp immunity, pulp innervation, and tubular dentin formation [11]. Dentin/pulp regeneration is feasible using DPSCs and SHEDs, both of which are safe and have promising benefits. Amongst the dental-derived stem cells, SHEDs are the most accessible ones as they cause no tooth pain but offer humongous regenerative abilities [12]. Regeneration of the pulp-dentin complex is a huge challenge, and this is where SHED enhances the possibility for better life of the tooth and facilitates better aspects of tooth regeneration [12]. Removing the necrotic pulp tissues was the conventional strategy followed for the treatment of an infected root pulp and replacing them with bioinert cement to block the root canal [1]. While this procedure sounds simple and effective, the only downside is the inability to restore the lost dental pulp tissue and the tooth's vitality [1].

A study by Miura et al. Showed that transplanting SHEDs with hydroxyapatite/tricalcium phosphate (HA/TCP) led to dentin formation in immune-compromised mice, and even this did not happen across all species under research. [13] A couple of other studies showed that SHEDs transplanted with hydrogel, collagen type 1, or PPLA in immune-compromised mice led to the formation of pulp-like tissues, including blood vessels and odontoblasts [14, 15]. Total pulp regeneration with neurogenesis and vasculogenesis was achieved in an adult canine using pulpectomy and pulp transplantation [17]. Various results proved one fact—complete regeneration of the dentin-pulp complex in vivo wasn't successful [16].

The differentiation of SHEDs into odontoblasts in human root canals was discovered by Rosa et al., and such odontoblasts regenerated dentin [18]. Currently, local regenerative pulp treatments using vital pulp can regenerate only the dentin-pulp complex without sufficient blood supply. Making use of modern tissue engineering that recaps the amputated pulp in combination with scaffolds, stem cells, and growth factors enables pulp tissue regeneration alongside the collateral blood supplement dentin-like hard tissue formation [5]. Total pulp regeneration with vasculogenesis and neurogenesis was successful in an adult canine model with pulpectomy and pulp transplantation. Likewise, using mobilized DPSCs to pulpectomized dog teeth showed pulp regeneration without any adverse effects with the treated teeth showing pulp recovery [1]. A study by Rosa et al. showed that transplanting SHED into human root canals produced functional odontoblasts capable of regenerating tubular dentin [14]. Pulp revascularization could become possible in the future using a combination of disinfection and debridement of infected root canal systems alongside the use of stem cells, growth factors, and scaffolds [1]. Various ongoing studies also offer the hope of viability restoration in the necrotic young permanent tooth with the production of the new dental pulp.

A study by Khorsand et al. that transplanted DPSCs into a canine periodontitis model showed successful cementum and PDL tissue regeneration [11]. DPSCs elevated the human salivary gland (HSG) differentiation into functional gland tissues when mixing and transplanting them into immunocompromised mice [11].

Another study by Dissanayaka et al, showed that spheroids of DPSCs prepared using umbilical vein stem cells and implanting them with a tooth slice in immunodeficient mice resulted in pulp-like tissue regeneration [11]. Various studies show that DPSCs are better for dentin/pulp regeneration complex compared to SHED as better clarity is needed on SHED's features to incorporate them in the clinical setting [11].

Dentin formation alongside pulp-like tissue occurrence was evident in immune-deficient mice when DPSCs were used in combination with HA/TCP [19-24]. DPSCs, in combination with fibrin/TCP, resulted in the formation of dentin-like tissue when transplanted into the renal capsule of mice [25, 26]. We also have research clearly showing the importance of growth factor (GF) concentrations in the dentin matrix as these GFs come in direct contact with the pulp and hence, in turn, affect DPSC proliferation during the initial stages of pulp repair [27]. Wnt3A, bFGF, SDF-1, and G-CSF are some of the prominent GFs that showcase the proliferative effect on DPSCs [11].

Periodontal Tissue Regeneration

WHO statistics show that at least 10-15% of the world population is affected by advanced periodontitis [28]. Clinicians have achieved successful results using various surgical procedures, and the discovery of stem cells could be an excellent tool for periodontal regeneration in the coming years. But the very high prevalence of periodontitis and PDL's role in maintaining tooth health has turned our attention toward the engineering of the PDL tissue [1]. Periodontal ligament (PDL) is the highly specialized connective tissue that encircles the root of teeth contributing toward ingraining the teeth in the jaw, feeding, repairing, and harbouring progenitor cell groups called as PDLSCs and maintaining tooth homeostasis. [29]. One of the latest reports founds that PDLSC-implanted mice were able to generate cementum-like structures that imbibe the characteristics of natural PDL [29,30]. Such results show clear evidence for the possibility of the formation of periodontal structures in which the cementum and PDL are included as well. Some factors affecting the regenerative capacity of the PDLSCs are the scaffolds that help in transplantation. The first transplantation of the human PDLSCs on a scaffold from HA/TCP was done on animal models resulting in the regeneration of cementum and PDL-like structures [31]. Such positive results were a motivating factor for other researchers to use PDLSCs in many other animals, including mice, rats, dogs, and many more, combining with different biomaterials as scaffolds (such as gelatin sponge, HA disks, β-tricalcium phosphate/type I collagen, and nerve fibers) to regenerate the periodontium tissues successfully [32-34].

Such positive outputs suggested that it's possible to generate cementum-like tissues and dentin/pulp-like structures using dental bud cells (DBCs), autologous or allogenic PDLSCs grafts, etc. When the appropriate scaffolds are used, the initial cell concentration is optimized [32, 35] Dental tissue stem cells are currently being used in cell transplantation therapies for correcting dentin and periodontal disorders. We have a study showing that the efflux of fluorescent dye Hoechst 33342 fetched from the human dental pulp cells and periodontal stem cells fetched from human extracted teeth led to partial regeneration of dentin and periodontal tissues in adult teeth. [2] Recreating a new PDL after the in-vivo implantation of dental follicle stem cells has also been successful. [1] Continuous efforts are still taken to come up with the perfect delivery system that could help in achieving a good cell-based therapeutic tool for regenerating periodontal tissue [36].

Whole Tooth Regeneration

Tooth loss not only affects an individual physically but also compromises the quality of life of the patient ruining their mental health as well. While this is a primary contributing factor, the regeneration of various tooth elements motivates scientists to delve into the aspect of entire tooth regeneration [5]. Any tooth is a combination of both hard and soft tissues, with the enamel, cementum, and dentin comprising the vascularized hard tissues surrounding the dental pulp. Building a tooth (in vivo or in vitro) needs the association of the odontogenic mesenchymal cells and epithelial cells [37]. Any bioengineered tooth should include root generation and PDL containing the nerve and blood supplies. Any whole tooth regeneration using tissue engineering follows one of the two methods, namely the scaffold method or the recombined tooth germs/cell aggregates method [1]. The scaffold method makes use of stem or precursor cells having a proper spatial orientation with the help of collagen sponge scaffolds or polymer membranes that are biodegradable [38, 39]. However, various research has reported a higher success rate when using collagen sponge scaffolds, in comparison to a biodegradable polymer [39]. Also, the sequential arrangement of the epithelial and mesenchymal cells within the scaffold enhances the tissue arrangement of the bioengineered teeth [40].

In the recombined tooth germs method, tooth formation relies upon using the DMSCs and the dental epithelial stem cells (DESCs) that are then transplanted into the alveolar bone to enable further eruption and growth from the gingiva [5]. Combining the extracted dental epithelial tissue and the mesenchymal cells of the cell pellet isolated from the E13.5 mice molar tooth germ, the artificial tooth germ developed and, as a result produced the desirable tooth structure by transplantation into the subrena capsule [41]. Tooth generation resulted along with periodontal-like tissue formation, owing to the regeneration of the tooth germ between the dissociated epithelial and mesenchymal cells of the E14.5 molar germ [42, 43]. It's also been proved that artificial tooth root formation is possible by combining the dental pulp stem cells and the periodontal ligament stem cells placed on separate scaffolds [44]. The latest development is a novel bioengineered method whose scope could be extended to growing various tooth types, such as molars and incisors [2].

The topmost hurdle in establishing successful dental regenerative therapies is nurturing the growth of bioengineered tooth germ in an adult environment [2]. However, recent research showed that a bioengineered tooth germ primordium isolated from a bioengineered tooth germ has the potential of growing into a tooth having the dentin, enamel, root, periodontal ligament, dental pulp, alveolar bone, and blood vessels in the tooth cavity after extracting the mandibular incisor [45].

Limitations

On one hand, stem cell-based regenerative therapy seems to hold the key to the repair of defective functions and tissues with the use of appropriate strategy, but being a relatively new area of research, dental pulp stem cells in regenerative therapy face various hurdles and risks. For instance, some of the primary parameters, including stem cell density and availability, besides making use of the right strategies need to be chosen carefully [1]. Besides these, a very important concern among dentists is dealing with immunity-related issues and controlling infections during cell transplantations, root canal treatments, and intracanal medicaments [4]. Ensuring complete dentin formation that covers the regenerated pulp is another challenging issue to deal with to prevent microleakage [4]. Immune rejections during cell transplantation must be dealt with tactically with maximum biosecurity. Cell-based medicinal products don't guarantee sterilization, removal/inactivation of the virus, and purification and if proper care isn't taken, any transfer of viral, bacterial, or fungal pathogens could prove to be life-threatening [46,47].

Conclusions

Exponential research on stem cell therapies for human use is happening continuously, but the progress of such therapeutic applications is proceeding slowly. Research on dental regenerative medicine gives us hope for organ replacement besides stem cell transplantation, but we are yet to reach a stage wherein the patient's cells could be used to stay out of any rejections. Dental-origin stem cells have eminent potential for multi-purpose applications but come with certain limitations currently, as a major portion of the research is still confined to animal models. We need vigorous and extensive clinical testing and research to enable the same in humans. Though we have reached a stage where the production of a teeth-like structure is possible, this cannot replace the actual tooth as more research is needed to grow the nerve and blood supply of teeth, for enabling complete tooth functionality. Constant evolution in the tissue engineering methodology coupled with a better understanding of DSC biology could provide the perfect platform for better use of DSC in regeneration therapy. The next decade could be a crucial junction where huge leaps in stem cell-based regenerative therapies could become a reality with successful tissue engineering therapies, this could be a biological alternative to synthetic materials that are in use currently. But dental stem cells have their share of challenges for which the research must happen effectively adhering to social responsibilities at all levels.

Conflict of Interest

There is no conflict of interest.

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References

[1] Chalisserry, E. P., Nam, S. Y., Park, S. H., & Anil, S., 2017, Therapeutic potential of dental stem cells. *Journal of Tissue Engineering*, 8, 2041731417702531.

[2] Nakao, K., & Tsuji, T., 2008, Dental regenerative therapy: Stem cell transplantation and bioengineered tooth replacement, *Japanese dental science review*, 44(1), 70-75.

[3] Nakashima, M., & Akamine, A., 2005, The application of tissue engineering to regeneration of pulp and dentin in endodontics. *Journal of Endodontics, 31*(10), 711–718.

[4] Nakashima, M., Iohara, K., Murakami, M., Nakamura, H., Sato, Y., Ariji, Y., & Matsushita, K., 2017, Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. Stem cell research & therapy, 8(1), 61.

[5] Soudi, A., Yazdanian, M., Ranjbar, R., Tebyanian, H., Yazdanian, A., Tahmasebi, E., Keshvad, A., & Seifalian, A., 2021, Role and application of stem cells in dental regeneration: A comprehensive overview. *Excli Journal*, *20*, 454–489.

[6] Bansal, R., & Jain, A., 2015, Current overview on dental stem cells applications in regenerative dentistry. *Journal of Natural Science, Biology, and Medicine*, *6*(1), 29–34.

[7] Mason, C., & Dunnill, P., 2008. A brief definition of regenerative medicine. Regenerative Medicine, *3*(1), 1–5.

[8] Gronthos, S., Mankani, M., Brahim, J., Robey, P. G., & Shi, S., 2000, Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America, *97*(25), 13625–13630.

[9] Panitvisai, P., & Messer, H. H.,1995, Cuspal deflection in molars in relation to endodontic and restorative procedures. *Journal of endodontics,* 21(2), 57–61.

[10] Schwartz, R. S., & Robbins, J. W., 2004, Post placement and restoration of endodontically treated teeth: a literature review. *Journal of Endodontics,* 30(5), 289–301.

[11] Yoshida, S., Tomokiyo, A., Hasegawa, D., Hamano, S., Sugii, H., & Maeda, H., 2020, Insight into the Role of Dental Pulp Stem Cells in Regenerative Therapy. Biology, *9*(7), 160.

[12] Guo, R., & Yu, J., 2022, Multipotency & immunomodulatory Benefits of Stem Cells from Human Exfoliated Deciduous Teeth: Front. Dent. Med, 3, 805875.

[13] Miura, M., Gronthos, S., Zhao, M., Lu, B., Fisher, L. W., Robey, P. G., & Shi, S., 2003, SHED: stem cells from human exfoliated deciduous teeth. Proceedings of the National Academy of Sciences of the United States of America, *100*(10), 5807–5812.

[14] Rosa, V., Zhang, Z., Grande, R. H., & Nör, J. E., 2013, Dental pulp tissue engineering in full-length human root canals. *Journal of Dental Research, 92*(11), 970–975.

[15] Cordeiro, M. M., Dong, Z., Kaneko, T., Zhang, Z., Miyazawa, M., Shi, S., Smith, A. J., & Nör, J. E., 2008, Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. *Journal of Endodontics*, *34*(8), 962–969.

[16] Zhai, Q., Dong, Z., Wang, W., Li, B., & Jin, Y., 2019, Dental stem cell and dental tissue regeneration. *Frontiers of Medicine*, *13*(2), 152–159.

[17] Iohara, K., Imabayashi, K., Ishizaka, R., Watanabe, A., Nabekura, J., Ito, M., Matsushita, K., Nakamura, H., & Nakashima, M., 2011, Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived

factor-1. Tissue engineering. Part A, 17(15-16), 1911–1920.

[18] Rosa, V., Zhang, Z., Grande, R. H., & Nör, J. E., 2013, Dental pulp tissue engineering in full-length human root canals. *Journal of dental research*, *92*(11), 970–975.

[19] Gronthos, S., Mankani, M., Brahim, J., Robey, P. G., & Shi, S., 2000, Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(25), 13625–13630.

[20] Gronthos, S., Brahim, J., Li, W., Fisher, L. W., Cherman, N., Boyde, A., DenBesten, P., Robey, P. G., & Shi, S., 2002, Stem cell properties of human dental pulp stem cells. Journal of dental research, 81(8), 531–535.

[21] Wang, J., Ma, H., Jin, X., Hu, J., Liu, X., Ni, L., & Ma, P. X., 2011, The effect of scaffold architecture on odontogenic differentiation of human dental pulp stem cells. *Biomaterials*, *32*(31), 7822–7830.

[22] Wang, J., Ma, H., Jin, X., Hu, J., Liu, X., Ni, L., & Ma, P. X., 2011, The effect of scaffold architecture on odontogenic differentiation of human dental pulp stem cells. *Biomaterials*, *32*(31), 7822–7830.

[23] Batouli, S., Miura, M., Brahim, J., Tsutsui, T. W., Fisher, L. W., Gronthos, S., Robey, P. G., & Shi, S., 2003, Comparison of stem-cell-mediated osteogenesis and dentinogenesis. *Journal of dental research*, *82*(12), 976–981.

[24] Sun, H. H., Chen, B., Zhu, Q. L., Kong, H., Li, Q. H., Gao, L. N., Xiao, M., Chen, F. M., & Yu, Q., 2014, Investigation of dental pulp stem cells isolated from discarded human teeth extracted due to aggressive periodontitis. *Biomaterials*, *35*(35), 9459–9472.

[25] Chun, S. Y., Lee, H. J., Choi, Y. A., Kim, K. M., Baek, S. H., Park, H. S., Kim, J. Y., Ahn, J. M., Cho, J. Y., Cho, D. W., Shin, H. I., & Park, E. K., 2011, Analysis of the soluble human tooth proteome and its ability to induce dentin/tooth regeneration. *Tissue engineering. Part A*, *17*(1-2), 181–191.

[26] Takeda, T., Tezuka, Y., Horiuchi, M., Hosono, K., Iida, K., Hatakeyama, D., Miyaki, S., Kunisada, T., Shibata, T., & Tezuka, K., 2008, Characterization of dental pulp stem cells of human tooth germs. *Journal of dental research*, *87*(7), 676–681.

[27] Moussa, D. G., & Aparicio, C., 2019, Present and future of tissue engineering scaffolds for dentin-pulp complex regeneration. *Journal of tissue engineering and regenerative medicine*, *13*(1), 58–75.

[28] Petersen, P. E., & Ogawa, H., 2005, Strengthening the prevention of periodontal disease: the WHO approach. *Journal of periodontology*, *76*(12), 2187–2193.

[29] Seo, B.M., Miura, M., Gronthos, S., Bartold, P.M., Batouli, S., Brahim, J., Young, M., Robey, P.G., Wang, C.Y., Shi, S., 2004, Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet, 364: 149-155.

[30] Shi, S., Gronthos, S., Chen, S., Reddi, A., Counter, C. M., Robey, P. G., & Wang, C. Y., 2002, Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression. *Nature biotechnology*, *20*(6), 587–591.

[31] Aoki, A., Mizutani, K., Schwarz, F., Sculean, A., Yukna, R. A., Takasaki, A. A., Romanos, G. E., Taniguchi, Y., Sasaki, K. M., Zeredo, J. L., Koshy, G., Coluzzi, D. J., White, J. M., Abiko, Y., Ishikawa, I., & Izumi,. Y.,2015, Periodontal and peri-implant wound healing following laser therapy. *Periodontology 2000*, *68*(1), 217–269.

[32] Tsumanuma, Y., Iwata, T., Washio, K., Yoshida, T., Yamada, A., Takagi, R., Ohno, T., Lin, K., Yamato, M., Ishikawa, I., Okano, T., & Izumi, Y., 2011, Comparison of different tissue-derived stem cell sheets for periodontal regeneration in a canine 1-wall defect model. *Biomaterials*, *32*(25), 5819–5825.

[33] Han, J., Menicanin, D., Marino, V., Ge, S., Mrozik, K., Gronthos, S., & Bartold, P. M., 2014, Assessment of the regenerative potential of allogeneic periodontal ligament stem cells in a rodent periodontal defect model. *Journal of periodontal research*, *49*(3), 333–345.

[34] Ninomiya, T., Hiraga, T., Hosoya, A., Ohnuma, K., Ito, Y., Asashima, M., & Nakamura, H.,2014, Enhanced Bone-Forming Activity of Side Population Cells in the Periodontal Ligament. *Cell Transplantation*. 2014;23(6):691-701.

[35] Kuo, T.F., Huang, A.T., Chang, H.H., Lin, F.H., Chen, S.T., Chen, r.S., Chou, C.H., Lin, H.C., Chiang, H., & Chen, M.H., 2008, Regeneration of dentin-pulp complex with cementum and periodontal ligament

formation using dental bud cells in gelatin-chondroitin-hyaluronan tri-copolymer scaffold in swine. Journal of Biomedical Materials research. Part A. 2008 Sep;86(4):1062-1068.
[36] Robey, P.G., & Bianco, P., 2006, The use of adult stem cells in rebuilding the human face. J Am Dent Assoc, 137(7): 961–972.
[37] Aly L. A., 2015, Stem cells: Sources, and regenerative therapies in dental research and practice. *World journal of stem cells*, *7*(7), 1047–1053.
[38] Duailibi, M. T., Duailibi, S. E., Young, C. S., Bartlett, J. D., Vacanti, J. P., & Yelick, P. C., 2004, Bioengineered teeth from cultured rat tooth bud cells. *Journal of dental research*, *83*(7), 523–528.
[39] Sumita, Y., Honda, M. J., Ohara, T., Tsuchiya, S., Sagara, H., Kagami, H., & Ueda, M., 2006, Performance of collagen sponge as a 3-D scaffold for tooth-tissue engineering. *Biomaterials*, *27*(17), 3238–3248.
[40] Honda, M. J., Tsuchiya, S., Sumita, Y., Sagara, H., & Ueda, M., 2007, The sequential seeding of epithelial and mesenchymal cells for tissue-engineered tooth regeneration. *Biomaterials*, *28*(4), 680–689.
[41] Yamamoto, H., Kim, E. J., Cho, S. W., & Jung, H. S., 2003, Analysis of tooth formation by reaggregated dental mesenchyme from mouse embryo. *Journal of electron microscopy*, *52*(6), 559–566.
[42] Hu, B., Nadiri, A., Bopp-Kuchler, S., Perrin-Schmitt, F., Wang, S., Lesot, H., 2005, Dental epithelial histo-morphogenesis in the mouse: positional information versus cell history. Arch Oral Biol, 50, 131-136.
[43] Hu, B., Nadiri, A., Kuchler-Bopp, S., Perrin-Schmitt, F., Peters, H., & Lesot, H., 2006, Tissue engineering of tooth crown, root, and periodontium. *Tissue engineering*, *12*(8), 2069–2075.
[44] Sonoyama, W., Liu, Y., Fang, D., Yamaza, T., Seo, B. M., Zhang, C., Liu, H., Gronthos, S., Wang, C. Y., Wang, S., & Shi, S., 2006, Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PloS one*, *1*(1), e79.
[45] Nakao, K., Morita, R., Saji, Y., Ishida, K., Tomita, Y., Ogawa, M., Saitoh, M., Tomooka, Y., & Tsuji, T., 2007, The development of a bioengineered organ germ method. *Nature methods*, *4*(3), 227–230.
[46] Herberts, C. A., Kwa, M. S., & Hermsen, H. P., 2011, Risk factors in the development of stem cell therapy. *Journal of translational medicine*, *9*, 29.
[47] Saini, R., Saini, S., & Sharma, S., 2009, Stem cell therapy: the eventual future. *International journal of trichology*, *1*(2), 145–146.