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"Stem Cell-Based Strategies for Dental Tissue Regeneration: Advances, Molecular mechanisms, and Future Perspectives"

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ABSTRACT:

Stem cell therapy for dental tissue regeneration is another innovation in regenerative dentistry providing new treatment methods for dental tissue defects caused by a disease or injury. This review gives a concise description and comparison of the various stem cells that are useful in dental tissue engineering; dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), and periodontal ligament stem cells (PDLSCs). The review presents the main aspects of stem cell-mediated tissue repair and regeneration, such as signalling pathways, the role of extracellular matrix (ECM), and scaffold biomaterial effects. Recent clinical uses of tissue engineering and stem cells in the dental field including pulp/periodontal regeneration right up to total tooth complex bioengineering are reviewed. Furthermore, the review explicates the issues and concerns linked with stem cell therapies and also discusses the future research that may bring about the improvement of stem cell therapies in terms of efficiency and safety. The areas where CRISPR, 3D bioprinting, and nanotechnology are incorporated are also considered to discuss their applicability in dental tissue regeneration.

- Stem Cells
- Dental Tissue Regeneration
- Dental Pulp Stem Cells (DPSCs)
- Periodontal Ligament Stem Cells (PDLSCs)
- Tissue Engineering
- Regenerative Dentistry
- Biomaterial Scaffolds
- Clinical Applications
- CRISPR
- 3D Bioprinting

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Introduction

Even with the progress in conventional methods of dental treatment, the problem of replacement of lost dental tissues due to caries, periodontal diseases, trauma or birth abnormalities has dwelled. Biomedical approaches of dentistry including dental filling, root canal treatment, tooth implantation, etc aims at merely treating the signs and symptoms and replacing the tissues and organs physically, but fail to reconstruct the functional and biological features of native teeth. Due to this limitation, the concept of regenerative dentistry has been established where efforts are made to replace lost dental tissues through advanced bio-sciences, for instance stem cell therapy.

Dental tissue regeneration can be achieved by stem cells since they have the capability of self-renewal and differentiation into specific cell types. Out of all the various stem cells, dental pulp stem cells (DPSCs) , stem cells of human exfoliated deciduous teeth and periodontal ligament stem cells have been seen to hold promising prospects for dental tissue engineering. DPSCs, procured from dental pulp tissue, equally possess the ability to osteogenic potential and convert into odontoblast-like cells which are capable of forming a dentin-like matrix. This process is controlled by fundamental signaling molecular pathways – the BMP pathway for instance is acknowledged to have a distinct responsibility in odontoblastic differentiation and dentinogenesis^1,2. Also, transforming-growing factor-beta (TGF-β) plays an essential role in the maintenance of the stemness of the DPSCs and differentiation into odontoblast-like cells ^3.

Based on stem cells from human exfoliated deciduous teeth (SHED), SHEDs have greater proliferative and differentiation potential as compared to DPSCs. Derived from SHED, studies have demonstrated that these cells are capable of giving rise to mesodermal derivatives such as osteoblasts and adipocytes with antigenic evidence of alkaline phosphatase and collagen type I^4. They have been shown to possess abilities of engaging in bone formation with, for instance, SHED having the capacity to differentiate into bone cells and promote the growth of de novo bone tissue in animal models of bone injury^5.

Odontoblast-like cells are derived from periodontal ligament stem cells (PDLSC) and are important for periodontal tissue regeneration. They can also undergo differentiation into cementoblasts, osteoblasts and fibroblasts which means that they play a role in the synthesis of cementum, alveolar bones and the fibres of the periodontal ligament. PDLSCs interaction with scaffold and growth factors enhance the regeneration of periodontal tissues. For instance, the analyzed research indicated that the use of platelet-derived growth factor-(PDGF) and bone morphogenetic proteins (BMP) stimulated PDLSCs be was effective in periodontal tissue engineering ^ 7.

The present pilot investigations and future clinical trials indicate that stem cell-based therapy is an effective mean for dental regeneration. For instance, a recent clinical study on the patients with necrotic teeth also showed the ability of DPSCs to regenerate pulp successfully and also the vitality and function of pulp were maintained after treatment ^8. In a similar manner, research work using other biomaterial scaffolds like bioactive glasses and calcium phosphate-based materials, the results of stem cell-mediated tissue regeneration have been enhanced due to the supportive biomaterial scaffold during cell attachment and differentiation ^9.

However, these are several issues that researchers are still facing in taking stem cell therapies from the lab to the patients. It was the case of questions regarding stem cell procurement, proliferation and differentiation as well as examination of safety and efficiency of the therapies. Finally, ethical concerns especially on the exploitation of embryonic stem cells as well as the possibility of forming tumor are challenges that ethical issues raise^10.

Since these diseases are prevalent in the entire world, these require some interventions. It is estimated that dental caries are present in about 2. 4 billion people round the globe, and periodontal disease affects approximately 10-15 percent of the global population ^11. This high prevalence of dental conditions stresses the demand of higher level regeneration solutions that target the biological flaws and offers maintenance and functional fix.

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The present review is intended to encompass generalized methodologies on stem cells based dental tissue regeneration. It will also cover the molecular processes that underlie stem cell development and tissue generation, brief on the existing therapies and applications, highlight the issues, and consider prospects of the escalating field. Combining the current scientific literature with clinical information, thereby aiming to provide the reader with state-of-the-art knowledge of how stem cell therapies are envisioned to revolutionise dental practice and the quality of care.

Stem cells Relevant to Dental Regeneration :

The various stem cell-based therapies have been revealed to have limitless prospect in dental tissue engineering where cell material characterization holds the prospect of replacing damaged tissues thanks to the fact that stem cells have the ability to transform themselves into different types of cells that are a core part of the dental structure. From all the durable stem cell varieties studied, DPSCs, SHED, PDLSCs and bone marrow-derived mesenchymal stem cells (BM MSCs) received considerable interest. Given characteristics of each of these stem cell types is different, thus they are suitable in several ways for dental tissue regeneration.

Primary cells of interest : Dental Pulp Stem Cells (DPSCs)

DPSCs, initially characterized by Gronthos and colleagues in the year 2000, are MSCs originally derived from dental pulp of both the deciduous as well as the permanent teeth^1. One of the important characteristics of DPSCs is their capacity to differentiate into odontoblast like cells required for dentinogenesis. Though the direct participation of DPSCs in odontogenesis has not been investigated, the function of those sustainable cells is regulated mainly by exciting routes such as bone morphogenetic proteins (BMPs) and transforming growth factor-beta (TGF-β) . BMP-2 for instance has been found to harbour odontoblastic differentiation where the level of dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP1) have been found to be high^3.

This is due to the fact that DPSCs have high proliferation rate, as well as the data on in vivo the ability of generate dentin-pulp complexes. A recent study has also shown that when DPSCs are seeded on the scaffold and implanted into immunocompromised mice the cells can form a pulp-dentin complex with functional vasculature and hence the cells have significant application in regenerative endodontics ^5. However, the markers are expressed in the DPSCs which includes STRO-1, CD146, CD44, which is variance with its multilineage differentiation potentials^6. They have also been demonstrated to produce several growth factors, including the vascular endothelial growth factor (VEGF) and the nerve growth factor (NGF) that promote vascularisation and neuronal differentiation in the regenerated tissue ^7^.

Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

SHED, initially identified by Miura et al. in 2003 are MSCs isolated from the dental pulp of sloughed deciduous teeth [8]. While DPSCs, SHED is shown to have a higher rate of proliferation, as well as better strength of differentiation for osteogenic and dentinogenic lineages^9. SHED have been observed to give early signals of osteoblast differentiation; alkaline phosphatase (ALP) and collagen type I essential in the formation of bones and dentine^10. Furthermore, SHED produce a higher amount of osteogenic factors such as BMP-2 and BMP-7 much higher than DPSCs^11.

In several animal models, SHED has been shown to have potential of dental tissue regeneration. While SHED transplanted to immunocompromised mice had the ability to form dentin-like extracellular matrix and even eliciting formation of new blood vessels in the pulp tissue^12. Further, SHED have been employed in the models of craniofacial bone defect where they provided a bone deposit in critical size lesion^13. These data provide evidence that SHED is a easily harvestable and fairly rich source of stem cells for applications in regenerative dentistry.

Periodontal Ligament Derived Stem Cells (PDLSCs)

PDLSCs disclosed by Seo et al. in 2004 are a kind of MSCs derived from periodontal ligament (PDL) tissue. These cells are very important for the homeostasis of periodontal tissues such as cementum, alveolar bone and PDL. It has been reported that PDLSCs can also

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give rise to cementoblasts, osteoblasts and/or fibroblasts and they play a very important role in periodontal tissue regeneration^15. PDLSCs differentiation is controlled by Wnt/β-catenin, BMP and TGF-β pathways, that interferes with the osteogenic and cementogenic ability of the cells^16.

Several in vitro investigations have also revealed that the PDLSCs possess the osteogenic differentiation potential with regard to ALP, RUNX2 and osteocalcin. Furthermore, PDLSCs present a high cloning efficiency and maintain MSC-related markers STRO-1, CD146, and CD105^18. In vivo, the PDLSCs promote the regeneration of periodontal ligament-like tissue, and form new fibrils in cementum and alveolar bone in the animal models of periodontal diseases^19. In addition, since PDLSCs do promote the regeneration of lost periodontal tissues, they have been applied in periodontal clinical trials, and resulted in better clinical outcome^20.

Mesenchymal stem cells derived from bone marrow (BM-MSC)

BM-MSCs which are derived from bone marrow are considered to be one of the most extensively researched in the field of regenerative medicine. These cells have been under a lot of research and studies of their abilities for tissue engineering of several tissues including dental tissues. Based on the discussed research BM-MSCs are multipotent or even pluripotent cells that can differentiate into the cells of osteogenic, chondrogenic, and adipogenic lineages^21. To the extent of dentin and bone formation, human BM-MSCs exhibit odontoblast-like cells and when placed in the defective teeth area^22.

Indeed, the pathways that could be associated with the differentiation of BM-MSC into dental tissues are BMP; Wnt/β-catenin and Notch^23. For instance, BMP-2 in the study by had the capability of promoting odontogenic markers including DSPP and DMP1 in the BM-MSCs hence formation of dentin like structures. Furthermore, it has been established that Wnt/β-catenin plays a role in the modulation of BM-MSC cycle and differentiation, studies have depicted that activation of Wnt/β-catenin pathway promotes the osteo/osteoblastic capacity of BM-MSCs^25.

The in vivo experiments have shown the potency of the BM-MSCs in dental tissue engineering. For instance, BM-MSCs when they were cultured on a scaffold and then transplanted to a rat model of periodontal disease were able to form alveolar bone and PDL-like tissue making a natural dentition periosteum^26. Also, BM-MSCs have applied in clinical studies for treating craniofacial defects and the applications have records or positive outcomes in bone formation and functional recuperation^27.

The Molecular Basis of Stem Cells in Dental Tissue Repair

Stem cell mediated dental tissue regeneration depends heavily on the multi-cell signaling mechanisms through which stem cells are guided on the pathways of differentiation, proliferation and tissue specific gene expression patterns. The following are some of the points that need to be properly understood to enhance the ability in stem cell mediated dental tissue engineering.

Bone Morphogenic Proteins (BMPs)

BMPs are a family of protein that is a member of the TGF-β super family of proteins that is critical as a signaling factor in the differentiation of stem cells into odontogenic and osteogenic lineages. BMP-2, BMP-4, and BMP-7 have been found to modulate odontoblast differentiation in DPSCs and BM-MSCs; therefore, those factors are the best candidates for the analysis of their effects on undifferentiated human BM-MSCs. BMP signaling activate Smad proteins through phosphorylation and translocation to nucleus and involved in the regulation of odontogenic markers such as; dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP1)^6. This proved the importance of BMP-2 in dentinogenesis as growing rodent teeth using BMP-2 augmented dentin formation on stem cell seeded scaffolds^7.

Wnt/β-Catenin Pathway

In addition, the Wnt/β-catenin pathway is also essential in the regulation of stem cell’s differentiation in dental tissue formation. Deregulation for Wnt pathway has been also reported to induce the differentiation of stem cells to osteoblasts and odontoblasts. Stimulated

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through the activation of BM-MSCs, Wnt signaling promotes the up regulation of RUNX2 as well as ALP that subsequently leads to bone and dentinogenesis^8,9. On the other hand, the antagonism of the Wnt pathway has been associated with the suppression of dentin formation and ability of DCSCs to mineralize^10. Its modulation is therefore, a potential approach of overcoming these difficulties hence this pathways modulation present a worthy approach to increasing the ability of the stem cells to repair and regenerate.

Notch Signaling Pathway

Notch signaling pathway is evolutionary, it takes part determination and the fate of stem cells in dental tissues. Ligation of Notch receptors includes Jagged1 and Delta1 to liberate the NICD that on translocating to the nucleus regulates the expression of genes underlying signaling^11. Protein Notch signaling has been proved to be involved in the self renewing ability of DPSCs and PDLSCs, also for their differentiation toward odontoblast or cementoblast control for special condition^12. Experiments have shown that regulation of Notch signaling can increase the abilities of these cells for regeneration, especially in periodontal and root formation^13.

Fibroblast Growth Factors (FGFs)

FGFs are another class of signaling molecules which has been investigated widely in terms of its association with stem cell-mediated regeneration. The members of the FGF family, with specific emphasis on FGF-2 and FGF-8, have been found to play a useful role in the regeneration and differentiation of dental stem cells, as well as the generation of new blood vessels which are essential for the survival and incorporation of the transplantation cells^14. FGFs act through the binding to tyrosine kinase receptors / activating intracellular pathways MAPK/ERK – followed by odontogenic, osteogenic gene transcription*15. For example, it was identified that FGF-2 stimulates the osteogenic differentiation of BM-MSCs by increasing the levels of RUNX2 and OCN^16 in the cells^16.

Hedgehog Signaling Pathway

Among the Hedgehog signaling proteins, Sonic Hedgehog (Shh) is particularly critical for the management of the dental stem cells. Shh signaling has been confirmed that it has a functional role in the tooth development, and has already been seen to modulate DPSCs ’proliferation and odontoblast differentiation ^[17]. The pathway works through interacting with the Patched receptor which otherwise inhibits the Smoothened protein, activation of Gli transcription factors to enable the synthesis of odontogenic genes^18. Abrogation of Shh signaling has therefore been implicated in aberrations of tooth morphogenesis and regeneration.

Discussion

Comparative Analysis of Stem Cell Therapies

Stem cell application in dental tissue engineering has been proved but there is a need to understand the comparative effectiveness of different stem cells. BM-MSCs have, therefore, been established to exhibit a high osteogenic and dentinogenic potential due to their totipotency in the generation of different cell phenotypes. However, MSCs in comparison to other stem cells such as SHED present with reduced ability to proliferate^1,2.

Unlike DPSCs and PDLSCs which have demonstrated better rejuvenative ability in their specialized realms, dentin and periodontium, respectively. In the research of Sakai et al., it was emphasized that PDLSCs exhibition greater periodontal regenerative capacity than BM-MSCs and this might be because of the fact that PDLSCs are derived from periodontal ligament tissue, thus have suitable environment. Recommended Further Reading Similarly, previous studies also demonstrated that SHED possesses a superior differentiation potential to differentiate into dentin-forming cells, probably due to greater proliferative potential and the presence of odontogenic genes including DSPP and BMP-2 ^4.

However, for comparative purposes, each stem cell type seems to have its ‘Special Strengths’; yet the type of stem cells to use for reconstructing any specific dental tissue needs to be informed by the targeted tissue type. In addition, the use of biomaterials such as the scaffold and growth factors can improve the reparative capability of these cells as noted from the various in vivo and clinical studies.^5 However, the major issue arises in an ability to quantify and standardize use of such treatments in mass production hospitals.

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Challenges and Limitations

Despite, the positive outcomes observed in preclinical studies, there are some concerns that need to be overcome to apply these findings in clinical settings. A major drawback, however, is the inability to control stem cell differentiation and the generation of functional dental tissues that closely resemble native tissues. However, there are still questions about the sustainability and functionality of the regenerated tissues, especially if one takes into consideration the oral cavity as a milieu that undergoes constant mechanical stress, as well as being exposed to microbes and immune cells^6.

Ethical issues are also an issue of debate especially in the application of extra-embryonic stem cells and induced pluripotent stem cells (iPSCs). While iPSCs has been proposed as a better option because they are pluripotent and also, does not have ethical complications, the problem of tumorigenesis is still a challenge when using iPSCs for differentiation into specific lineages. Secondly, both the cost and the technology involved in stem cell therapies are other issues that act as constraints to the implementation of stem cell therapy in the clinics.

Another compelling factor that hinders the use of stem cell therapies in clinical practice is the legal issues. The strict rules and regulations that surround cell-based therapies are a hindrance to clinical translation and commercialization and therefore result in slow provision of the therapies to patients^8. Thus, there is a need to have more efficient rules and requirements that will allow achieving the main goals – safety and innovative development.

Future perspectives and new treatment modalities

As for the present and future advancements in this field, there are several promising technologies that may help solve the issues with stem cell-based dental tissue regeneration. Some of the most promising techniques have been Gene editing such as CRISPR/Cas9, which has the capacity to increase the quality of stem cells by fixing genetic mistakes or adding desirable characteristics^9. These enhancements could result into more deterministic and optimal reparative performances.

Another technology that could be vital in the advancement of dental tissue engineering is the 3D bioprinting technology. Thus, by adjusting the positions of cells, growth factors, and bio-materials at the microscale, it is possible to build tissues that mimic natural tooth structures using 3D bioprinting techniques^10. This technology seems to be useful in enhancing the organization and several other characteristics of the supplied tissues as implanted in the body especially when there are certain clinical conditions. Further, the increasing attention to the specific needs of individuals under personalized medicine may pave way for the use of stem cell therapies that are derived from the patient's cells to avoid the problem of immune rejection and enhance the chances of a favorable outcome^11. Current clinical trials and research into stem cells are looking into these possibilities and the future outcomes could open up new ways of developing better forms of stem cell treatments in dentistry.

Conclusion

Stem cell therapy is considered as an emerging field in dentistry that aims to provide the concept for the regeneration of dental tissues and structures that cannot be regenerated by other conventional treatments. Though BM-MSCs, DPSCs, SHED, and PDLSCs exert their own kind of regenerative potentials, the kind of stem cell to be used depends on theparticular clinical background and the type of tissue to be regenerated.

On one hand, there have been considerable advances in the nature of inventions and innovative stem cell approaches in preclinical and clinical settings, however, there are several unmet needs such as the inability to control stem cell differentiation, lack of long term stability, bioethics and regulatory issues. Recent technologies like gene editing, 3D bioprinting and personalized medicine are viewed as having potential to eliminate these barriers and take the field to the next level. In the future, interdisciplinary cooperation and future research between researchers and clinicians, as well as between these representatives and the corresponding authorities, will be crucial for the implementation of these discoveries into everyday clinical practice. Thus, the enhanced stem cell applications will offer optimal solutions that help patients achieve maximum benefits regarding dental

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tissue regeneration and open a new chapter in regenerative dentistry.

This structure enables a critical assessment of the benefits and drawbacks of the different stem cells types, and it also presents the current issues and prospective of the stem cell-based dental tissue regeneration. In the conclusion, it is all summed up and the benefits of these therapies again affirmed with the caveat of existing challenges.

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