MANUSCRIPT Scientific Services...
www.manuscriptscientific.com

Journal of Stem Cell & Therapeutic Research

Review Article

Small-Molecules in Somatic Cell Reprogramming and Its Applications in Dentistry

Dhvani S Pandya, Nikhil Harikrishnan, Selvakumar Kritika, N Ravikumar, S Kavitha* and Sekar Mahalaxmi

Department of Conservative Dentistry and Endodontics, SRM Dental College, Ramapuram, SRM Institute of Science & Technology, Ramapuram Campus, Bharathi Salai, Ramapuram, Chennai 600 089, Tamil Nadu, India.

Corresponding author: S Kavitha, Faculty of Department of Conservative Dentistry and Endodontics, SRM Dental College, Ramapuram, Chennai 600 089, Tamil Nadu, India, Tel: +919940048022; E-mail: kavithasanjeev02@gmail.com

Abstract

Regenerative medicine is an interdisciplinary field that integrates engineering and life science principles to create new biological alternatives through which the tissue function can be restored, maintained, or improved. The ultimate goal of regenerative therapy is to develop fully functioning bioengineered organs which work in cooperation with surrounding tissues to replace organs that were lost or damaged as a result of disease, injury, or aging. Recently, a new alternative has come forward that bring about chemical reprogramming of cells using "Small-Molecule". These molecules have a small size which are rendered suitable for multiple site-specific therapies. These act on particulate genes involved in its specific function thereby helping in differentiation. The pulp-dentin complex due to its dynamic anatomical and physiologic constraints is one of the most challenging tissues to regenerate. "Small-Molecule" can enhance stem cell properties of mesenchymal stem cells (MSCs) derived from human dental pulp (hDPSCs), which have potential for numerous clinical applications thereby reducing stem cell variability and improving replicability. "Small-Molecule" can induce reparative dentin formation, help in preventive management of dental caries and can also cause disruption of oral biofilms. Natural small molecules can also act as inhibitors of coronavirus lipid-dependent attachment to host cells which could be a possible strategy for reducing SARS-COV-2 infectivity. Thus, the aim of this review is to discover the concept of Small-Molecule Cell Reprogramming in dentistry. This novel approach may help open a pathway to a new code of stem cell therapy using chemical compounds.

Keywords: Cell reprogramming, Regeneration, Small-molecules, Stem cells, Tissue engineering

INTRODUCTION

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes such that tissue/organ functions can be maintained, restored and improved [1]. It is a triad of employing stem/progenitor cells, scaffolds and growth factors to regenerate functional biological tissues [2].

STEM CELL BIOLOGY

Stem cell research has been one of the current topics for the scientific community, certainly due to the number of possible applications of stem cells in regenerative medicine. Within a decade, multiple approaches in this field have become 'the most promising' tools to make things possible which was once thought to be still far from possible [3]. Stem cells are defined as clonogenic cells exhibiting the capacity for self-renewal and multilineage differentiation. They are mainly of 2 types namely embryonic stem cells (ECSs) and adult stem cells. Pluripotent ESCs derived from the inner cell mass of mammalian blastocysts can be maintained indefinitely in culture [4,5]. But due to ethical dilemmas, an alternative source is sought.

GENETIC REPROGRAMMING OF STEM CELLS

2006 marks a keystone achievement in science, by professor Shinya Yamanaka's team at Kyoto University, Japan for "Genetically Reprogramming" adult cells like mouse fibroblasts to pluripotent stem cells. This was done by forced expression of transcription factors namely Oct4(O), Sox2(S), Klf4(K), c-Myc(M) (Yamanaka's OSKM factors). These induced pluripotent stem cells (iPSC), genetically and functionally, behaved similarly to ESCs [6]. This has triggered vigorous interest among scientists as they serve as a renewable source to differentiate into precursors of virtually any cell type [5].

Similarly, in dentistry, regeneration is attracting growing interest mainly because of its translational and promising therapeutic approach. Human dental pulp stem cells (hDPSCs) were the fundamental type of dental mesenchymal

Received: *February 02, 2022;* ***Revised:*** *February 21, 2022;* ***Accepted:*** *February 24, 2022*

Citation: Pandya DS, Harikrishnan N, Kritika S, Ravikumar N, Kavitha S, et al. (2022) Small-Molecules in Somatic Cell Reprogramming and Its Applications in Dentistry. J Stem Cell Ther Res, 1(1): 1-5.

stem cells (MSCs) isolated and characterized from dental tissues for regenerative purposes. It has been demonstrated to regenerate pulp and dentine in emptied root canal space in animal models. The incorporation of a prefabricated vascular network into bioengineered pulp tissue constructs has been the most favorable approach to promote blood flow into the transplanted tissues by anastomosis of the graft and the host tissue vascularization. Apart from regenerating pulp, many possibilities of MSCs versatility appear to be amplifying. Regardless of such captivating properties, the inevitable drawback is their limited tissue sources. Also, acquiring, transporting, processing and characterization of the tissue for clinical usage is a major effort [7,8].

Alternatively, ESCs and the iPSCs are the cellular building blocks of vascular tissue for the process of pre-vascularization. Though it significantly enhances functional blood flow and tissue survival upon transplantation, generation of these cells through "genetic reprogramming / OSKM approach" with retroviruses or lentiviruses has been noted with diverse challenges such as ethical issues, its low efficacy of stem cell transformation (only 0.2%), failure to differentiate into their final cell types, apoptosis and safety concerns of genomic instability [8]. Furthermore, the potential for cancerous tumor formation, inhibition of tumor suppressors, generation of epigenetic defects, possibilities of degenerative pathways, duration and cost-effectiveness seems to be other major concerns [9].

DIRECT CELL REPROGRAMMING

Nevertheless, to overcome the issues in "genetic reprogramming approach", breakthrough research in different perspectives to process cells with greater efficiency have been proposed. "Direct cell reprogramming", otherwise called "Trans differentiation", is reprogramming of one somatic cell type directly into another, without the need to go through an induced pluripotent state. The process of trans differentiation does not require cell division, hence it reduces the risk of tumor formation and mutations making it more applicable for clinical applications in contrast to genetic manipulation/iPSC reprogramming. It is capable of reprogramming cells that are abundant in the body into desired cell phenotypes bringing about tissue repair and restoring tissue functions [10].

CHEMICAL REPROGRAMMING WITH SMALL MOLECULES

Efforts to optimize this reprogramming system by the use of non-integrating vectors, non-viral gene delivery methods, cell membrane permeable proteins, growth factors and small-molecule biological compounds have shown potential to stimulate regeneration [11]. Growth factors were found to be associated with several shortcomings such as instability in *in vivo* conditions, molecular weight, immunogenicity, hardships in sterilization, difficulty in preparation and cost. Small-molecule compounds due to their inherent unique properties dominates over the others. This review will discuss the role of small- molecules in regenerative dentistry.

"Chemical biology", a recent research arena has emerged as a highly interdisciplinary research field, which focuses on elucidating the mechanisms of biological phenomena by the application of chemical tools and techniques, namely "Small-Molecule Compounds" and chemical libraries. Small-molecules are carbon-based low molecular weight compounds (<900 daltons) that are less than that of macromolecules such as DNA, RNA and proteins [12].

PROPERTIES OF SMALL MOLECULES

Owing to their small size, these molecules have the added advantage of passing through cell membranes to reach targets, rendering them suitable for many target-specific therapies by regulating their biological processes. They are more easily synthesized and play an important role in modifying cell behaviors [7,12]. The reversibility of their activity, the potential for modulating multiple specific targets within a protein family or across a different protein family adds up to their advantages [13]. These desirable properties of small-molecule compounds allow the production of competent phenotypes to target particular cells in a synergistically favorable manner and are reported to play an important role in cell reprogramming without the introduction of ectopic genes by forced expression of Oct4, Sox2, Klf4, and C-Myc (OSKM)factors [14,15].

HISTORY OF SMALL MOLECULES

The history of "chemically induced reprogramming" dates to over a decade ago where they have used retinoic acid and its derivatives to induce mouse embryonic stem cells to express various cell phenotypes. Interestingly one other Molecule namely 5 azacytidine, a potent DNA methylation inhibitor was first synthesized 40 years ago, which was known to possess anti-metabolic activity when tested against cancer cells. Hence, it was widely thought to step back and redirect the earlier/older methods rather than moving to seek new ways. Consequently, the use of chemically induced cell programming (CiPSCs) with the use of small-molecules has been a breakthrough with many successful examples [16].

Chemically induced cell programming (CiPSCs) has intrinsic advantages namely a) duration- the drugs usually reach their target sites rapidly and selectively; b) control- concentration of the drug can be easily varied to obtain the desired effect in the most efficient way; c) simple- chemical treatment is a very simple tool; d) low cost- drugs once identified, can be easily synthesized in larger scale; e) they are specific and reversible [15,16].

SMALL MOLECULE-BASED COMPOUNDS

Recent studies have shown that reprogramming could be achieved using cocktails of small-molecules that are comparatively safer to use [8]. The chemical reprogramming approach was first reported by Hou et al [16]. who described

that a cocktail of small-molecule compounds namely valproic acid, tranylcypromine, forskolin, 3-deazaneplanocinA, CHIR99021, E-616452, and TTNPB can induce somatic cells of the mouse into induced pluripotent stem cells (iPSCs) which are otherwise called as "chemical-induced pluripotent stem cells" (CiPSCs)?

For example, Oct4 (one of the transcription factors) is central to the machinery governing pluripotency and the precise expression level of Oct4 determines the fate of embryonic stem cells. Forskolin, 2-methyl-5-hydroxytryptamine, and D4476 act as "chemical substitutes" for Oct4. These small-molecules can enable reprogramming even in the absence of Oct4 using Oct4 promoter-driven green fluorescent protein expression on OG- mouse embryonic fibroblasts, with viral expression of Sox2, Klf4, and c-Myc. Thus, as a master switch governing pluripotency, Oct4 expression, which is kept repressed in somatic cells by multiple epigenetic modifications, is unlocked in chemical reprogramming by application of various small- molecules. This study thereby demonstrates that, somatic reprogramming toward pluripotency can be manipulated using only small-molecule compounds. It reveals that the endogenous pluripotency program can be established by the modulation of molecular pathways nonspecific to pluripotency via small-molecules rather than by exogenously provided "master genes" [17]. Added to these discoveries were the commonly used biological reagent, bromodeoxyuridine, Repsox, Retinoic acid and Dorsomorphin [15].

Maintaining the stemness of stem cells had always been tricky in stem cell related research. Interestingly, small-molecules prevented the differentiation of human ESCs which were otherwise difficult to maintain in an undifferentiated state in cultures. 6-bromoindirubin-3-oxime, pluripotin, and rapamycin have shown to express pluripotent genes and maintain the stemness of human embryonic stem cells (hESCs) [7].

It was also noted that pluripotent genes like NANOG, SOX2, and OCT4 were expressed in higher concentration in these cells.

APPLICATIONS OF SMALL MOLECULES IN MEDICINE

Importantly, the small-molecules have proven to be effective *in vivo* in regenerative medicine. Greco [18] investigated small-molecules cannabidivarin, selurampanel (BGG492) and ganaxolone in phase II clinical trials for the treatment of epilepsy and highlighted that cannabidivarin is as an efficient anticonvulsant. Also, selurampanel (BGG492) and ganaxolone has been reported to be the only neurosteroid that is effective and well tolerated for the treatment of refractory generalized or focal epilepsy both in adults and in children [18]. Sandborn [19] used tofacitinib an oral, small-molecule janus kinase inhibitor as induction and maintenance therapy for ulcerative colitis and concluded that in patients with moderately to severely active ulcerative colitis, tofacitinib was more effective as induction and maintenance therapy than placebo [19]. These results demonstrate an approach where drugs currently used in the medical field can be repurposed for potential dental applications.

APPLICATION OF SMALL MOLECULES IN DENTISTRY

Dental caries is the most common disease of the oral cavity affecting the majority of the population. Control of biofilm formation has been a critical strategy for the preventive management of dental caries. Antimicrobial small- molecules have shown their potential in the disruption of the oral biofilms. A newly designed small-molecule compound ZY354 was synthesized to evaluate for antimicrobial properties. It was noticed that the extracellular polysaccharides, streptococcus mutans and dead/live microbial ratio in multispecies biofilms were significantly reduced. Furthermore, ZY354 exhibited decreased demineralization activity at biofilm/enamel interface [20].

Mineral aggregates such as Mineral trioxide aggregate and Biodentine are the material of choice for regenerative dentistry and have been reported to aid the formation of tertiary dentine. But, unfortunately the deposition of dentine is not at the sites of damage, but rather internal in the pulp space. In addition, due to the non-biodegradability nature of these materials, the full mineral volume is never restored [21].

Small-molecule namely glycogen synthase kinase (GSK-3) antagonists namely CHIR999021 and tideglusib has been used for the treatment of Alzheimer's disease, and it is observed to exhibit therapeutic value for many neurodegenerative diseases [21].

DENTIN REMINERALIZATION USING SMALL MOLECULES

Recently, Neves [21] used a novel biological approach that can stimulate and mobilize resident stem cells in the tooth pulp for the formation of reparative dentine by the use of these GSK-3 inhibitors [21]. Low doses of small-molecule namely glycogen synthase kinase (GSK-3) antagonists CHIR999021 and tideglusib small-molecules which was used initially for the treatment of Alzheimer's disease, and which was observed to exhibit therapeutic value for many neurodegenerative diseases was explored in dentistry. Low doses of these small -molecules were delivered at the target site by incorporating it in biodegradable, clinically-proven collagen sponges. It was noted that by 6 weeks of treatment, as the carrier sponge degraded, the whole injury site from occlusal to pulp chamber roof was replaced with reparative dentine simultaneously. This simple, complete, effective natural tooth repair process using small-molecules could thus potentially provide a new approach for clinical tooth regrowth.

A significant role of GSK-3 antagonist is represented by its capacity to modulate human stem cells *in vivo*. GSK3 small-

molecule antagonists have shown to efficiently upregulate Wnt pathway, maintaining an undifferentiated phenotype of embryonic stem cells, and retains the pluripotent state-specific transcription factors Rex-1, Oct-3/4, and Nanog [22]. Wnt signaling pathway is an ancient and evolutionarily conserved pathway that regulates crucial aspects of cell fate determination, cell migration, cell polarity, neural patterning and organogenesis during embryonic development [23] The Wnt is secreted by glycoproteins and comprise a large family of nineteen proteins in humans playing a pivotal role in signaling regulation, function and biological output. The drug seems to act by activating this pathway of resident mesenchymal stem cells from the tooth pulp, thereby providing a potential route for enhancing natural repair of the dentine. The result, on condition of replicability on human subjects, may lead to a pharmacological treatment for cavities [21].

MECHANISM OF Wnt/β-CATANIN SIGNALING PATHWAY

The activation of Wnt/β-catanin signaling as a universal "immediate to early response" to tissue damage provides a potential route for enhancing natural repair by overstimulating this pathway. Wnt/β-catenin signaling has emerged as a major target in tissue regeneration and repair which can be stimulated in different ways. The simplicity of this approach makes it ideally translatable into a clinical dental product for treatments requiring dentine restoration and pulp protection that are currently treated with non-organic cements. Thus, the rationale behind this, is that, the addition of Wnt signaling agonists/ GSK3 inhibitors may provide an effective way to stimulate reparative dentine formation and restore lost dentine following caries removal with naturally-generated new dentine [21].

ROLE OF SMALL MOLECULES IN REGENERATION

Small-Molecules were demonstrated to enhance the properties of hDPSCs by promoting and increasing the stemness, by intensifying ESCs self-renewal and by stimulating multiple cellular signaling pathways. Chemically induced neural progenitor cells, neuron cells, Schwann cells, and cardiomyocyte like cells can be obtained from non-pluripotent human cell lines using different small-molecules cocktails [7].

Based on its applications in reprogramming protocols, these small-molecules are categorized into

1. Epigenetic modulators
2. Metabolic regulators
3. Signaling pathway regulators
4. Other factors that bring about the characteristics of the designated cell types [8].

Small-molecules change the epigenetic feature of the starting cells by activating or inhibiting the enzymes that alter DNA thereby improving the reprograming efficacy. Most widely used small-molecule for these purposes is valproic acid. It has been reported that small-molecule, Repsox by selectively inhibiting TGF-β signals, enhances the mesenchymal-to epithelial transition during reprogramming [24]. Polyl: C, a toll -like receptor agonist (toll-like receptor 3) activates innate immune signals and transdifferentiate fibroblasts into endothelial cells. Also, VEGF 165 small-molecule along with other activators, drives the endothelial differentiation of SCAPs, which was confirmed with the generation of tubular like structures at 12 h.

OTHER APPLICATIONS OF SMALL MOLECULES

Other than its use in regeneration, its extended application in various clinical conditions marks its competence. Small-molecular chemicals, such as flavonoids and terpenoids, have been investigated with a view to promote bone formation/ in osteoporosis. Quercetin and kaempferol, the most widely used flavonoids, were reported to stimulate alkaline phosphatase activity of MG-63 human osteoblasts. Besides flavonoids, other types of small-molecules, such as retinoids and amilorides, were also found to stimulate the differentiation of osteoblasts [25].

Coming to the current global scenario that created a havoc in the field of medicine, the entry of COVID-19, was declared a pandemic in early 2020. It is caused by a pathogen namely, severe acute respiratory syndrome coronavirus 2 (SARSCoV2). Despite the availability of FDA-approved vaccines in early 2021, the pandemic remains the world's number one public health threat, with significant social and economic consequences. Viral infectivity generally depends on interactions between components of the host cell plasma membrane and the virus envelope. Cholesterol and lipid membrane rafts on the target cell is important for SARS-CoV2 infection, playing a key role in viral entry into the cell. Molecular inhibitors such as methyl-β-cyclodextrin (MβCD) and other small compounds with depletive cholesterol activity have been used to inhibit attachment of coronaviruses to host cells. Thus, this small molecule inhibitor therapy, slightly and dose-dependently reduced expression of ACE2 in the cell membrane, thereby reducing the infectivity of coronaviruses, such as SARS-CoV2 [26].

Hence small-molecules serve as good therapeutic option due to their ability to pass across cell membranes and reach target. This approach bypasses the high risks and high level of economical and time investment that are usually required in novel drug discoveries.

CONCLUSION

The fast progress in the field of chemical-mediated reprogramming/trans differentiation with the use of small-molecules provides us new ways to manipulate the cell fate in the human body and in the near future. Hence the chemical strategy could be another choice in the field of regeneration in addition to the cell replacement therapy. However, more

research will be necessitated to evaluate the genomic integrity of the cells generated with chemical-mediated reprogramming using small-molecule compounds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Ahmed GM, Abouauf EA, AbuBakr N, Dorfer CE, El-Sayed KF (2020) Tissue engineering approaches for enamel, dentin, and pulp regeneration: An update. Stem Cells Int 25: 5734539.
2. Langer R, Vacanti JP (1993) Tissue engineering. Science 260: 920-926.
3. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z (2019) Stem cells: Past, present, and future. Stem Cell Res Ther 10: 1-22.
4. Romito A, Cobellis G (2016) Pluripotent stem cells: Current understanding and future directions. Stem Cells Int 2016: 9451492.
5. Yen AH, Yelick PC (2011) Dental tissue regeneration-a mini-review. Gerontology 57: 85-94.
6. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663-676.
7. Al-Habib M, Yu Z, Huang GT (2013) Small molecules affect human dental pulp stem cell properties via multiple signaling pathways. Stem Cells Dev 22: 2402-2413.
8. Yi B, Dissanayaka WL, Zhang C (2020) Growth Factors and Small-molecule Compounds in Derivation of Endothelial Lineages from Dental Stem Cells. J Endod 46: S63-S70.
9. de Lázaro I, Cossu G, Kostarelos K (2017) Transient transcription factor (OSKM) expression is key towards clinical translation of *in vivo* cell reprogramming. EMBO Mol Med 9: 733-736.
10. Grath A, Dai G (2019) Direct cell reprogramming for tissue engineering and regenerative medicine. J Biol Eng 13: 1-5.
11. Xie X, Fu Y, Liu J (2017) Chemical reprogramming and trans differentiation. Curr Opin Gen Dev 46: 104-113.
12. Egusa H, Saeki M, Doi M, Fukuyasu S, Matsumoto T, et al. (2010) Small-molecule approach to bone regenerative medicine in dentistry. J Oral Biosci 52: 107-118.
13. Birjandi AA, Suzano FR, Sharpe PT (2020) Drug Repurposing in Dentistry; towards application of Small Molecules in Dentin Repair. Int J Mol Sci 21: 6394.
14. Qin H, Zhao A, Fu X (2017) Small molecules for reprogramming and trans differentiation. Cell Mol Life Sci 74: 3553-3575.
15. Anastasia L, Piccoli M, Garatti A, Conforti E, Scaringi R, et al. (2011) Cell reprogramming: A new chemical approach to stem cell biology and tissue regeneration. Curr Pharm Biotechnol 12: 146-150.
16. Anastasia L, Pelissero G, Venerando B, Tettamanti G (2010) Cell reprogramming: Expectations and challenges for chemistry in stem cell biology and regenerative medicine. Cell Death Differ 17: 1230-1237.
17. Hou P, Li Y, Zhang X, Liu C, Guan J, et al. (2013) Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science 341: 651-654.
18. Greco M, Varriale G, Coppola G, Operto F, Verrotti A, et al. (2018) Investigational small molecules in phase II clinical trials for the treatment of epilepsy. Expert Opin Investig Drugs 27: 971-979.
19. Sandborn WJ, Ghosh S, Panes J, Vranic I, Wang W, et al. (2014) A phase 2 study of tofacitinib, an oral Janus kinase inhibitor, in patients with Crohn's disease. Clin Gastroenterol Hepatol 12: 1485-1493.
20. Zhang C, Kuang X, Zhou Y, Peng X, Guo Q, et al. (2019) A novel small molecule, ZY354, inhibits dental caries-associated oral biofilms. Antimicrob Agents Chemother 63: e02414-e02418.
21. Neves VC, Babb R, Chandrasekaran D, Sharpe PT (2017) Promotion of natural tooth repair by small molecule GSK3 antagonists. Sci Rep 7: 1-7.
22. Sato M, Tada N (1995) Preferential expression of osteocalcin-related protein mRNA in gonadal tissues of male mice. Biochem Biophys Res Commun 215: 412-421.
23. Komiya Y, Habas R (2008) Wnt signal transduction pathways. Organogenesis 4: 68-75.
24. Heng BC, Jiang S, Yi B, Gong T, Lim LW, et al. (2019) Small molecules enhance neurogenic differentiation of dental-derived adult stem cells. Arch Oral Biol 102: 26-38.
25. Kim JG, Son KM, Park HC, Zhu T, Kwon JH, et al. (2013) Stimulating effects of quercetin and phenamil on differentiation of human dental pulp cells. Eur J Oral Sci 121: 559-565.
26. Baglivo M, Baronio M, Natalini G, Beccari T, Chiurazzi P, et al. (2020) Natural small molecules as inhibitors of coronavirus lipid-dependent attachment to host cells: A possible strategy for reducing SARS-COV-2 infectivity? Acta Bio Medica: Atenei Parmensis 91: 161.