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Review article

Dental applications of induced pluripotent stem cells and their derivatives☆

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ABSTRACT

Periodontal tissue regeneration is the ideal tactic for treating periodontitis. Tooth regeneration is the potential strategy to restore the lost teeth. With infinite self-renewal, broad differentiation potential, and less ethical issues than embryonic stem cells, induced pluripotent stem cells (iPSCs) are promising cell resource for periodontal and tooth regeneration. This review summarized the optimized technologies of generating iPSC lines and application of iPSC derivatives, which reduce the risk of tumorigenicity. Given that iPSCs may have epigenetic memory from the donor tissue and tend to differentiate into lineages along with the donor cells, iPSCs derived from dental tissues may benefit for personalized dental application. Neural crest cells (NCCs) and mesenchymal stem or stomal cells (MSCs) are lineage-specific progenitor cells derived from iPSCs and can differentiate into multilineage cell types. This review introduced the updated technologies of inducing iPSC-derived NCCs and iPSC-derived MSCs and their application in periodontal and tooth regeneration. Given the complexity of periodontal tissues and teeth, it is crucial to elucidate the integrated mechanisms of all constitutive cells and the spatio-temporal interactions among them to generate structural periodontal tissues and functional teeth. Thus, more sophisticated studies *in vitro* and *in vivo* and even preclinical investigations need to be conducted.

Contents

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1. The challenge of tumorigenicity in induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are derived from adult somatic cells by exogenously introducing different combinations of transcription factors, including *Oct3/4*, *Sox2*, *Klf4*, *c-Myc*, *Nanog*, and *Lin28* [1–3]. Human iPSCs (hiPSCs) are capable of multilineage differentiation and cause less ethical issues than human embryonic stem cells (hESCs), therefore, they are promising for the generation of patient- and disease-specific pluripotent stem cells to investigate disease mechanisms, screen drugs, and regenerate cells, tissues, and organs [2,4]. However, there are still many challenges to translate the promises into clinical application. In this current review, we would like to focus on the major challenge, tumorigenicity.

Over the past years, the risk of tumorigenicity of iPSCs is a significant concern [5,6], which mainly result from three factors including undifferentiated and/or immature cells in the final cell products that differentiate from the iPSCs, tumorigenic property of reprogramming factors in the iPSCs, and genetic mutations of iPSCs during in vitro culture [7]. Even very small amount of residual iPSCs or lineage-specific stem cells in the transplant could result in formation of teratoma or other tumors. It was shown that iPSCs do not contain more genomic variations than the fibroblast subclones by using whole exome sequencing and targeted resequencing, suggesting that iPSC reprogramming itself is not mutagenic [8]. However, the fact that gene expression networks in the induction of pluripotency interconnected with those in oncogenesis is evidenced by the common features of these genes, which possess high proliferation ability, self-renewal, DNA repair checkpoint disconnecting and capability to differentiated into varieties of tissues [6]. Thus, the methods to eliminate or reduce tumorigenicity may pave the way for clinical application.

1.1. Purification of differentiated cells

To reduce the risk of teratoma, transplantation of purification of differentiated target cells is feasible. Mandai et al. have reported highly efficient purification methods (specification > 95%) by checking pigmentation and immunostaining of retinal pigment epithelial markers BEST1 and PAX6, which showed negative result of in vivo tumorigenicity [9]. A clinical trial found that positively sorted hiPSCs-derived dopaminergic progenitor cells with CORIN maker survived and functioned as midbrain dopaminergic neurons in a primate model of Parkinson's disease [10]. Besides, Brentuximab vedotin that is approved in treatment of CD-30 positive lymphomas was used to eliminate the undifferentiated iPSCs, in which the CD30 marker is positive [11]. Both negative and positive cell sorting were performed to purify corneal epithelial cells by using antibodies targeting CD200, ITGB4 and SSEA-4 [12,13].

Even though the undifferentiated iPSCs were thoroughly eliminated, tumors may emerge from differentiated progeny cells that still have proliferative capacity [7]. Functional recovery was observed in the animal models of spinal cord injury after transplantation of the hiPSCs-derived neural progenitor cells (NPCs) [14]. However, the paralysis appeared again due to proliferation of nestin-positive NPCs when using some hiPSC lines [15]. By inhibiting notch signaling, which is important for self-renewal of NPCs, Okubo et al. succeeded to suppress tumorigenicity caused by immature NPCs [16].

1.2. Elimination of viral integration and substitution of reprogramming factors

The canonical virus-based delivery system cause problems, including gene integration with the host genome, insertional mutagenesis, apoptosis, cell senescence, and strong immunogenicity [17]. To solve these problems, many groups have focused on transferring reprogramming factors using non-integrating vectors or approaches, including adenoviral vector [18], plasmid [19,20], and lentiviral vector that can be excised after iPSC generation [21], and direct delivery of reprogramming RNA [22], and protein transduction via soluble antibodies [23] etc.

In addition, all the reprogramming factors inducing cell pluripotency are responsible for oncogenesis, especially the oncogene c-Myc, which is considered as a driver mutation in many human malignancies. A few compounds can replace one or more Yamanaka factors and facilitate the reprogramming of somatic cells into iPSCs, including valproic acid that dramatically increases reprogramming efficiency by almost 50–100 fold and maintains the iPSC condition without the oncogene *c-Myc* [24], kenpaullone that functionally substitutes oncogene *KlF4* to induce pluripotency [25], an inhibitor of transforming growth factor β (TGFβ) receptor I kinase (activin-like kinase 5, AlK5) that facilitates the efficiency of iPSC induction and replaces either *Sox2* or *c-Myc* with other three remaining factors rather than both of them concurrently [26], and inhibitors of the Src family kinases that functionally induce pluripotency in the absence of *Sox2* [27]. The methods that generate iPSCs by using only chemical compounds or molecules may open new avenues for the clinical application of iPSCs [28,29].

Each of these tactics has distinct reprogramming efficiency rate. The overviews of different iPSC reprogramming technologies are available [17,30,31]. In our institute, Center for iPS Cell Research and Application (CiRA), we routinely generate iPSCs with five-factor plasmids (pCE-hSK, pCE-hUL, pCE-hOCT3/4, pCE-mp53DD, and

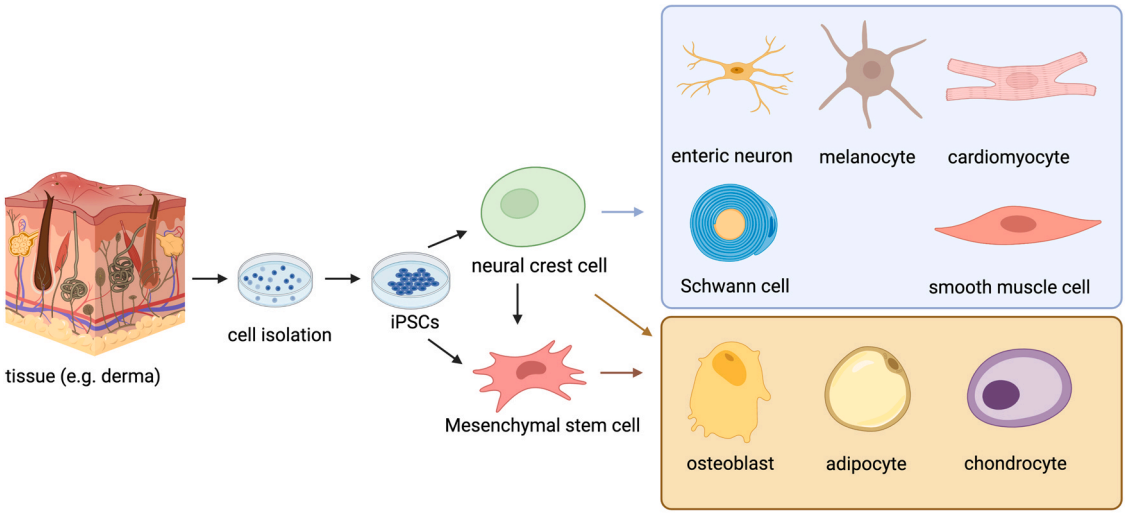

Fig. 1. The multiple cell lineages differentiated from iPSCs iPSCs, induced pluripotent stem cells.

pCXB-EBNA1), even for clinical use (https://www.cira-foundation.or.jp/e/project/homozygous.html).

1.3. Application of iPSC derivatives

It was indicated that the application of lineage-specific progenitor cells derived from iPSCs restricts the tumorigenicity of iPSCs [32]. Neural crest cells (NCCs) and mesenchymal stem or stomal cells (MSCs) can differentiate into multilineage cell types. NCCs are a transient group of cells from the ectoderm germ layer with multipotency to differentiate into various cell types, including MSCs, smooth muscle cells, enteric neurons, melanocytes, Schwann cells, osteoblasts, chondrocytes, adipocytes, and myocytes (Fig. 1) [33]. Therefore, NCCs are considered promising for craniofacial regenerative medicine. Induced-MSCs (iMSCs) derived from iPSCs exhibited similar tumor tropism, but less capacity of tumor promotion compared with bone marrow MSCs (BMSCs) [34]. Additionally, NCCs and MSCs can be populated in a feeder-free culture system. Moreover, the low immunogenicity of iPSC-derived NCCs on both molecular and functional levels indicates their promising clinical application [35].

1.3.1. Technologies to generate induced-NCCs (iNCCs) from ESCs or hiPSCs

Mizuseki et al. first generated iNCCs by treating stromal cell-derived inducing activity (SDIA; coculture on PA6 stromal cells)-treated mouse and primate ESCs with BMP4 after the fourth day of the coculture [36]. Jiang et al. cocultured the hESCs on PA6 feeder layer with SDIA and induced hESCs into iNCCs within one week [37]. Lee et al. isolated iNCCs from the neural rosette stage of hESCs and induced iNCCs into peripheral neurons and Schwann cells in vitro, and smooth muscle, adipogenic, osteogenic and chondrogenic cells in vivo [38]. The neural crest markers, p75 and HNK1, were used to isolate and enrich iNCCs using fluorescence-activated cell sorting (FACS) technology [37,38]. Small molecular compounds or recombinant proteins were used to induce differentiation of hESCs and hiPSCs into iNCCs, the underlying mechanisms of which were attributed to the synergistic suppression of SMAD signaling via two inhibitors, Noggin and SB431542 [39], or the activation of Wnt signaling and inhibition of Smad pathway simultaneously [40]. Menendez et al. further improved the protocol utilizing small molecular inhibitors of glycogen synthase kinase 3 (GSK3) and TGFβ for iNCCs generation and dramatically increased the enrichment efficiency to more than 90% without using feeder cells and a FACS sorting system [41]. Our group ever optimized the induction procedures of iNCCs derived from hiPSCs using a combination of GSK3β and TGFβ inhibitors [42]. Similarly, synchronous Wnt activation (via GSK3β suppression) and TGFβ inhibition (SB431542) induced three hiPSC lines into iNCCs [43]. The iNCCs stably maintained the gene expression profile as NCCs within 10 passages. James et al. robustly induced hESCs and hiPSCs into neural crest with a fully defined and xeno-free system combined with top-down inhibition of BMP4, which indicated that intermediate levels of BMP activity are necessary for human neural crest induction in vitro [44]. However, the characteristics of iNCCs changed gradually from pre-migratory to migratory during passage [45].

To further promote efficiency, Leung et al. reported a fast approach that generated iNCCs through the activation of WNT/β-Catenin pathway within five days [46]. It was evidenced that the reduced 2-day-pulse WNT activation induced by GSK3 inhibition optimized the generation of iNCCs [47]. The technologies of deriving iNCCs from hESCs and iPSCs are summarized in Table 1.

Although many groups have been trying to optimize the differentiation procedures of iNCCs [48,49], there are still some unsolved issues: (1) the accurate characterization of iNCCs using specific markers to isolate an identical NCC population; (2) the precise control of cell fate into iNCCs and not into other cell lineages; (3) the multipotency or stemness maintenance after long-term passages; (4) methods to obtain more cell lineages that are beyond cranial limitation and can be derived from iNCCs, such as endocrine cells, mesenchymal precursor cells, cardiac cells, and sympathoadrenal cells [50]; (5) the comparison of the gene expression profiles of iNCCs generated from distinct protocols.

1.3.2. Modulation of NCCs differentiation

In the developing embryo, the differentiation of NCCs is regulated by many transcription factors and molecules [51]. *Sox2*, *Sox5*, and *Sox10* contribute to neurogenic differentiation [51]. *Sox5* and *Sox9* promote chondrogenesis, while *Sox8* inhibits osteoblast differentiation [51]. The local environment can facilitate NCC differentiation into neural lineages and mesenchymal lineages in vitro under specific conditions. The scaffold's stiffness may partially explain NCC differentiation [52]. When NCCs are embedded in the nanofibrous vascular scaffold for rat vascular graft transplantation, smooth muscle cells and glial cells are induced near the outer space of the polymer grafts and the hydrogel, respectively. Transplanted NCCs could repair diabetic polyneuropathy in streptozotocin-diabetic mice, which indicates that paracrine actions may induce

Table 1
Induction of NCCs from iPSCs.

Reference	Cell source	Molecular Markers	Technology	Cell Lineages	Time length
[36]	mouse and primate ES cells	Ncx, Snail, Slug, dHand, Msx1	Late BMP4 exposure of SDIA-treated ESCs	autonomic, sensory, and smooth muscle cell lineages	4–10 days
[38]	hESCs	p75, HNK1, AP2, CD49d	Isolation from the neural rosette stage of hESCs via FACS with cell coculture	Peripheral neurons, Schwann cells, smooth muscle cells, mesenchymal precursors, osteocytes, chondrocytes, adipocytes	6–8 weeks
[37]	hESCs	p75	Coculture of hESCs on PA6 fibroblast feeder layer with stromal-derived inducing activity	Peripheral nerves, glia, myofibroblasts	1 week
[39]	hESCs, hiPSCs	p75, HNK1, AP2, PAX7	Due inhibition of SMAD signaling via Noggin and SB431542	Pigmented cells, dopaminergic cells, motoneuron	11-day
[40]	hESCs, hiPSCs	p75, HNK1, AP2	Agonist of Wnt signaling inhibitor Smad pathway	Peripheral neurons, smooth muscle cells, mesenchymal cells, osteocytes, chondrocytes, adipocytes	12–14 days
[41]	hESCs, hiPSCs	p75, HNK1, AP2, FoxD3	Inhibitors of GSK3 and TGFb	Peripheral neurons, MSCs, osteocytes, chondrocytes, adipocytes	15-day
[42]	hESCs, hiPSCs	p75, TFAP2A	Inhibitors of GSK3 and TGFb with insulin	Peripheral neurons, glia, melanocytes, corneal endothelial cells, MSCs, osteocytes, chondrocytes, adipocytes	7-day
[46]	hESCs, hiPSCs	SOX10, PAX7, TFAP2A	Activation of WNT via CHIR 99021 (a GSK3 inhibitor)	Peripheral neurons, glia, chondroblast, osteoblasts, melanoblasts	5-day
[44]	hiPSCs	P75, HNK1, TFAP2α, PAX3, SOX10	Culture with NCN2 medium containing N2 supplement, CHIR99021, and SB431542, with top-down inhibition of BMP4	Peripheral neurons, sensory neurons, and glial cells	7-day
[43]	hiPSCs	HNK1, P75, PAX3, ZIC1, SOX9/10, FOXD3, PAX6, ETS1	Synchronous Wnt activation (via GSK3b suppression) and TGFb inhibition (SB431542)	Mesenchymal progenitors with potential osteogenic and chondrogenic differentiation	35–40 days
[47]	hESCs	SOX10, PAX7	2-day pulse activation of WNT/ b-CATENIN via GSK3 inhibition	peripheral neurons, glia, melanoblasts, ectomesenchymal osteocytes, chondrocytes, and adipocytes	2-day

transplanted NCCs into mesenchymal and neural derivations concomitantly in vivo [53]. Moreover, the potential regulatory mechanism is associated with various genes and epigenetic modifications. The activation status of TGFβ and its concomitant effect on SOX10 are associated with the differentiation into mesenchymal or neural derivatives from NCCs in vitro and in vivo [54]. The increased expression of miR-21 promotes hair follicle-derived NCCs differentiation into Schwann cells by downregulating Sox2 expression in vivo and in vitro [55]. Noisa et al. evidenced that blocking Notch signaling inhibited the formation, migration, and differentiation of premigratory neural-crest-like cells from hESCs [56]. The neural differentiation from PDL-derived NCCs is associated with TNF signaling, VEGF signaling, and nectin adhesion pathways [57]. Low-intensity pulsed ultrasound promotes iNCCs to differentiate into neural derivatives by modulating angiogenesis and nervous system-related genes [58].

Overall, NCCs differentiate into neural and mesenchymal lineages in vitro and in vivo under genomic and epigenetic modification and the effect of the local microenvironment.

1.3.3. Generation and characterization of iPSC- and NCC-derived MSCs

The minimal criteria of human MSCs formulated by the International Society of Cellular Therapy include plastic adherence under standard culture conditions; ≥ 95% of MSCs expressing CD105, CD73, and CD90 markers and ≤ 2% expressing CD45, CD34, CD14 or CD11b, CD79 or CD19, and HLA-DR; properties to differentiate into osteoblasts, adipocytes, and chondrocytes [59]. In our opinion, genome-wide expression and DNA methylation profiles were suggested for analysis in animal experiments and preclinical trials [60].

MSCs derived from bone marrow, adipose tissue, or other connective tissues have limited proliferative capability, the phenotype and differentiation of which may change in the long-term culture [61]. Moreover, the donor's age and culture passages result in replicative senescence and impair the regenerative potential of MSCs [61,62]. With infinite self-renewal, broad differentiation potential, and less ethical issues than embryonic stem cells, iPSCs are promising progenitor cells to generate iMSCs.

The strategies of generating iMSCs mainly consist of dissociating iPSC colonies, the formation of embryoid bodies (EBs), and induction using fibrillar collagen or small molecular inhibitors [63–70]. In a study, iPSCs cultured on synthetic polymer-coated plates with human-cell-conditioned medium supplemented with fibroblast growth factor 2 (FGF2) were cultured in suspension to form EBs; the EBs were then seeded on the gelatin-coated dishes with growth medium to form fibroblastic cells in morphology; finally the MSC makers, including positive CD166, CD105, CD90, and CD73, and negative CD31, CD34, and CD45 were identified by using FACS [67]. Similarly, a cardiomyogenic medium with a p38-MAPK inhibitor was used to derive EBs from iPSCs; then the parallelly differentiated contracting cardiomyocytes (CMs) and MSCs were separated; CD44, CD105, CD90, and CD29 were used to distinguish CMs and MSCs in the EBs [68].

FGF2, platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) have been used in research to differentiate iPSCs into MSCs; $CD24^{-}CD105^{+}$ and green fluorescence protein positive (GFP^{+}) have served as cell sorting makers and colony screening labels [63,69]. MSC induction media comprising of DMEM-HG, 10% defined fetal bovine serum, 1% nonessential amino acids, 1% penicillin-streptomycin, and 5 ng/mL human recombinant FGF2 were used to induce MSCs differentiation from iPSCs, subsequently positive makers (CD29, CD44, CD73, CD90, CD105, CD166, and HLA-ABC) and negative makers (HLA-DR, CD31, and CD45) were detected as mesenchymal phenotypes of MSCs [70]. Human ether-à-go-go 1 (hEAG1) channel is key in regulating the proliferation rate of iMSCs [69].

It was reported that hESCs and iPSCs cultured on type I collagen coated plates with αMEM medium supplemented with 10% FBS, 50 μM magnesium L-ascorbic acid phosphate, 100 nM dexamethasone and ROCK inhibitor Y-27632 are singly dissociated, forming fibroblast-like cell colonies after a 10-day culture [66]. Positive CD73, CD90, CD105, CD146 and CD166 and negative CD34 and CD45 were used to characterize MSCs [66]. It was reported that cultures in MSC media have shown that hESCs and hiPSCs treated by a TGF-β inhibitor (SB431542) express decreased pluripotency-associated genes of OCT4 and LEFTY1/2, and increased mesodermal related genes of MSX2, NCAM, and HOXA2, with differentiation into MSCs [65].

Established protocols have been reported for the efficient generation of MSCs from iNCCs. In studies, MSC media with or without supplements were used to induce MSCs from iNCCs [71,72]. $LNGFR^{+}THY\text{-}1^{+}$ NCLCs derived from hESC and hiPSCs had similar potential as MSCs to differentiate into mesenchymal lineages [73]. Our group succeeded in obtaining MSCs from iNCCs by culturing in the αMEM medium supplemented with 10% FBS and analyzing CD73, CD44, CD45 and CD105 as MSC markers [42].

2. iPSCs in dentistry

2.1. iPSC generation from dental tissues

The tissue surrounding teeth is called periodontal tissue and consists of alveolar bone, periodontal ligament (PDL), and gingiva. Given the convenient accessibility and acquisition, dental tissues are promising cell sources for iPSC generation. Until now, iPSCs have been successfully generated from diverse dental tissues, including dental pulp, oral mucosa, gingiva, and PDL [32]. We herein focus on the details of the reprogramming methods and the efficiency of generating iPSCs derived from different dental tissues (Table 2). Notably, the proliferative ability of donor cells is positively associated with the reprogramming efficiency of iPSCs [74]. Although the cellular origin also affects the differentiation potentials of iPSCs, which accounts for the transient epigenetic memory of the donor origin in early-stage iPSCs, these marks largely disappear upon passaging [75]. iPSCs derived from specific dental tissues, especially from the patients' own tissues, may benefit in personalized dental application.

2.2. Dental applications of iPSCs

The addition of iPSCs with a silk scaffold and enamel matrix derivatives (EMD) promotes alveolar bone, cementum, and PDL formation [76]. iPSCs with EMD or growth/differentiation factor-5 (GDF-5) in vitro dramatically up-regulate the gene expression of bone, PDL, and cementum with regard to periodontal tissues [77]. iPSCs and bone morphogenetic protein 6 (BMP-6) promote bone and cementum generation, while hydrogel-encapsulated iPSCs-BMP-6 decrease the inflammatory cytokine levels and facilitate bone and PDL regeneration [78]. Human gingival fibroblasts (hGFs)-derived iPSCs with hydrogel have increased mineralized structure generation and higher expression of periodontal associated markers [79]. The ease of accessibility, high proliferation, and differentiation capability of iPSCs makes them an alternative cell source for tooth regeneration. Wen et al. established a tooth germ model consisting of dental epithelial and mesenchymal cells isolated from the surrounding tissues of the mandibular incisor of ED14.5 mice and MEF-derived iPSCs and transplanted the constructed tooth germs into subrenal capsules for 4 weeks. Bone-, dentin-, and pulp-like structures and osteopontin were detected using hematoxylin and eosin (HE) and immunohistochemical staining [80]. Tooth-like structures (dental pulp, dentin, enamel space, and enamel organ) and enamel-secreting ameloblasts have been established in mouse subrenal

Table 2
iPSCs derived from dental tissues.

Reference	Tissue source	Cell source	Technology	Vectors/ Molecules	Efficiency/Remarks
[126]	Gingiva of adult wild-type mouse or human	Mouse gingival fibroblasts (GFs)/primary human GFs (hGFs)	Expression of four factors (Oct3/4, Sox2, Klf4 and c-Myc) or three factors (without the c-Myc)	pMXs-IRES-puro	Four-factor reprogramming efficiency of mouse GFs was more than 7-fold higher than that of fibroblasts from tail-tips
[127]	Oral mucosa	Oral mucosa fibroblasts (OFs)	Retroviral transduction of OCT4, SOX2, c-MYC, and KLF4	pMIG-hOCT3/4, pMIG-hKLF4, pMIG-hSOX2, and pMXs-hc-MYC	The passage of one iPSCs costs 17–24 days of transduction
[74]	Pulp of human third molars	Mesenchymal stromal cells (MSCs)	Retroviral transduction of OCT3/4, SOX2, and KLF4 without MYC	pMXs-IRES2-DsRed-ENTR-D	MSCs from a 10-year-old donor had 30–100-fold higher efficiency than other clonally expanded MSCs and human dermal fibroblasts
[119]	Pulp of human third molars	Dental pulp cells (DPCs)	Retroviral transduction of OCT3/4, SOX2, c-MYC, and KLF4	pCRXL-TOPO-OCT3/4, pMXs-hSOX2, pMXs-hKLF4, and pMXs-hc-MYC	Dental pulp cells were suggested to be a prospective source of iPS cell banks in regenerative medicine
[128]	Pulp of exfoliated deciduous teeth, apical papilla, and dental pulp	Stem cells from exfoliated deciduous teeth (SHED), stem cells of the apical papilla (SCAP), and dental pulp stem cells (DPSCs)	Viral transduction of Lin28/Nanog/Oct4/Sox2 or c-Myc/Klf4/Oct4/Sox2	pLenti6.2/C-Lumio/V5-DEST-(c-Myc, Klf4, Oct4, or Sox2), pSin-EF2-gene-Pur-(Lin28, Nanog, Oct4, or Sox2), pMXs-(c-Myc, Klf4, Oct4, or Sox2)	The reprogramming of all three into iPSCs was suggested as having higher efficiency than fibroblasts
[129]	Human immature dental pulp	Human immature dental pulp stem cells (hIDPSCs)	Retroviral transduction of OCT4, SOX2, c-MYC, and KLF4	pMx vectors	The reprogramming of hIDPSCs was fast and able to generate iPSCs under feeder-free conditions.
[130]	Human gingiva and periodontal ligament (PDL)	hGFs, and human PDL fibroblasts (hPDLFs)	Retroviral transduction cocktail of OCT3/4, SOX2, KLF4 and c-MYC.	pLenti6/UbC/ mSlc7a1	The iPSCs derived from both hGFs and hPDLFs supply an alternative cell source to implement tissue-regenerative treatment for periodontitis
[77]	Human gingiva	GFs	Delivery of Oct4, Sox2, Klf4, L-myc, Lin28 and TP53 shRNA.	Episomal plasmid vectors	Integration-free and feeder-free approach decreases the risk of genetic mutation and increases efficiency without cell sorting

capsules by transplanting a recombinant unit of epithelial sheets differentiated from human urine induced pluripotent stem cells (hU-iPSCs) and the dental mesenchyme of E14.5 mouse [81]. The iPSCs derived from dental pulp stem cells (DPSCs) with poly-L-lactic acid scaffolds were transplanted into immunodeficient mice, afterwards, a structure of tubular dentin with a pulp-like tissue was formed [82].

Hertwig's epithelial root sheath (HERS) of the enamel organ is key in the formation of tooth roots [83]. Epithelial rests of Malassez (ERM) are epithelial remnants of HERS in the periodontium after tooth development [84]. Kim et al. immortalized epithelial-like stem cells derived from hiPSCs (EPI-hiPSCs) with HERS/ERM cell lines as feeder cells and demonstrated the amelogenic and odontogenic differentiation of EPI-hiPSCs by co-culturing them with hDPSCs [85]. Collectively, these studies suggest the promising potential of iPSCs in periodontal and tooth regeneration.

2.3. Dental iPSC-derivatives

2.3.1. Dental tissue derived iNCCs

Dental stem cells can come from different human dental cells such as postnatal DPSCs, stem cells from human exfoliated deciduous teeth (SHED), hSCAPs, dental follicle precursor cells (DFPCs), periodontal ligament stem cells (PDLSCs) and the cells of oral mucosa [86]. Dental tissues originate from the neural crest and can be used to generate neural crest like stem cells (NCLSCs) from dental tissue-derived iPSCs or DPSC [87–91]. Decreasing the fetal bovine serum concentration could increase the expression of neural crest markers, p75 and HNK-1, in SHED-derived dental stem cells [91]. A floating culture system is efficient in keeping DPSC stem properties and neural crest makers, nestin, CD271, and SOX-10 [90]. Additionally, PDL-derived iPSCs exhibit higher differential capability and more NCC-associated phenotypes and functional hallmarks than non-neural crest tissue-derived iPSCs, although they are both HNK-1-positive, which indicates the priority of dental tissues as NCC sources [92]. Genome-wide microarray analysis revealed that NCC-derived mesenchymal progenitors shared a high degree of similarity to dental stem/progenitor cell populations including DPSCs, hSCAPs, PDLSCs, and BMSCs [43]. However, reports on the efficiency of generating iNCCs from dental tissue-derived iPSCs or DPSC are scarce.

2.3.2. iNCCs and amelogenesis

The ameloblasts of the enamel organ contribute to enamel formation [93]. It is well known that mesenchymal cells, derived from the NCC precursor, differentiate into dental papilla cells and dental follicle cells (DFCs), which contribute to form the dentin-pulp complex, PDL, and alveolar bone [94]. Recently, cell-type-specific genetic tools that enable labeling, monitoring, and manipulation of the organ components consist of a powerful approach for the study of tooth development and tooth regeneration. Combined with other recombinases and transcriptional regulation system, the *Cre* driver transgenic mouse lines with *Cre* reporter lines have allowed more sophisticated control and tracing of specific cell population [95]. Through a *Irf6* (expressed throughout the embryonic ectoderm) conditional knockout mouse generated via a *Pitx2-Cre* driver line, Chu et al. found that *IRF6* has role in tooth number, crown and root morphology, and amelogenesis [96]. The *Wnt1-Cre* transgenic mice, known as an NCC-specific *Cre* mouse line, in combination with a *Cre* reporter line *R26R* reveals that NCCs contribute the formation of dentin-pulp complex rather than dental enamel in tooth development [97]. Although all these transgenic lines such as *P3Pro-Cre* [98] and *Ht-PA-Cre* [99], are reported to be NCC-specific, the expression patterns are different. Another NCC-specific Cre mouse line, P0-Cre, with R26R mice, indicate that NCCs are also responsible for the development of the enamel organ and enamel formation [100]. Moreover, DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) -labeled NCCs have been shown to be present in both the dental mesenchyme and enamel organ epithelium [101].

2.3.3. iNCCs and odontogenesis

Mouse iPSC-derived neural crest-like cells (iNCLCs) had a gene expression pattern associated with odontogenic mesenchymal cells; and the iNCLCs exhibited potential to differentiate into odontoblasts and dental pulp cells when cultured in conditioned medium of mouse dental epithelium cultures [102]. Seki et al. differentiated iPSC cell-derived neural crest-like cells (iNCLCs) into odontoblast-like cells by exogenously expressing Pax9 and Bmp4, the interaction of which regulated the extracellular matrix proteins, dentin matrix protein 1 (Dmp1), and dentin sialophos-phoprotein (Dspp), thereby regulating the mineralization function of odontoblast-like cells [103]. Notably, teratoma formation was not observed when iNCLCs and Pax9- and Bmp4-overexpressing iNCLCs were transplanted into mice, which indicated the safety without tumorigenicity of iNCLCs for tooth regeneration [103]. Mouse cranial neural crest cell line O9–1 and iNCLCs respectively differentiate into DMP-1^{+} odontoblasts in a developing tooth germ in vitro and form structural vascularized dentin-pulp complex with tooth scaffold in vivo [88].

2.3.4. iNCCs and cementogenesis

Cranial neural crest-derived cells (CNCCs) differentiated into cementoblast-like cells when incubated with a DFC-conditioned medium supplemented with dentin non-collagenous proteins [104]. These cementoblast-like cells are associated with high ALP activity and enhanced calcified nodule formation.

2.3.5. iNCCs and periodontal tissues and bone

iPSCs-derived NCLCs cultured on an extracellular matrix (ECM) were indicative of decreased ESC and NCC markers, but increased MSC and PDL-related markers, which suggests the potential application of iNCCs for PDL regeneration [105]. Neural crest stem-like cells derived from human gingiva-derived mesenchymal stem cells can differentiate into neuronal and Schwann-like cells and exhibit regenerative function in facial nerve defects in a rat model [106]. NCC-derived chondrocytes (NCC-Chs) and mesodermal cell-derived chondrocytes (MC-Chs) can form hyaline cartilage, with the former being more similar with native articular chondrocytes in terms of morphology and transcription than the latter [107].

Overall, iNCCs with dental epithelial cells are ideal cell resources for tooth regeneration. Given the structural complexity of the entire tooth, it is crucial to elucidate the mechanisms of iNCCs differentiation into different odontogenic cells spatially and temporally (Fig. 2).

2.3.6. Application of iMSCs

Dental tissue engineering relies on signaling molecules, cells, blood supply, and scaffolds [108]. iMSCs cultured on biofunctional calcium phosphate cement (CPC) have increased proliferation, actin fiber expression, and osteogenic differentiation and mineralization, which indicate that iPSC-MSC-CPC have potential for bone regeneration [109]. hiPSC-MSC like cells (hiMSCs) derived from gingiva and PDL can differentiate into osteoblasts, adipocytes, and chondrocytes in vitro; however, mineralized structures analogous to mature bone were only observed in PDL-derived hiMSCs when implanted into NOD/SCID mice in vivo [110]. In the first application of periodontal tissue regeneration using hiMSCs, which were implanted to the surgically modified alveolar bone defect on rats, the regeneration of fibrous tissue, mineralized tissue, and newly formed PDL-like tissue were promoted significantly [111]. The exogenous expression of TSG-6 in rat iMSCs dramatically attenuates the inflammatory reaction of experimental periodontitis and suppresses the osteoclast-associated alveolar bone resorption [112].

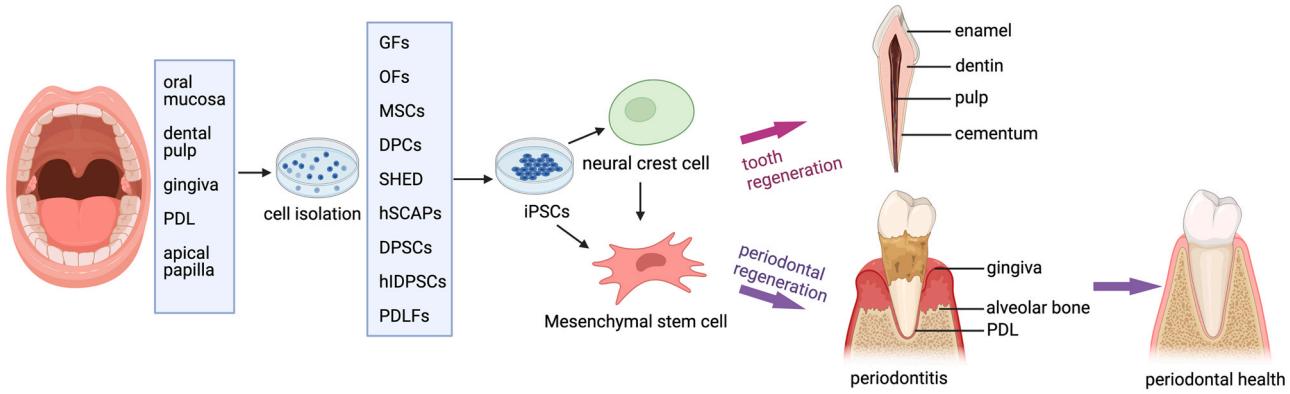

Fig. 2. Potential application of iPSCs, NCCs, and MSCs in periodontal and tooth regeneration GFs, gingival fibroblasts; OFs, Oral mucosa fibroblasts; MSCs, Mesenchymal stromal cells; DPCs, Dental pulp cells; SHED, Stem cells from exfoliated deciduous teeth; hSCAPs, stem cells from the apical papilla; DPSCs, dental pulp stem cells; hIDPSCs, Human immature dental pulp stem cells; PDL, periodontal ligament; PDLFs, periodontal ligament fibroblasts.

The iNCCs-derived MSC-like cells (iNCMSCs) that our group generated exhibited homogenous character and osteochondral differentiation in vitro, although they did not restore the osteochondral defects in rats [71]. However, in another study, thyroid cartilage was generated by transplanting clumps consisting of iMSCs via iNCCs intermediates and extracellular matrix complex into immunodeficient rats [72]. iMSCs may be promising cell sources for bone and cartilage regeneration with optimized induction conditions.

Macroporous CPC scaffolds support the adhesion and viability of iPSC-derived human mesenchymal progenitors and affect the expression of bone-related genes, thereby having potential in dentistry and orthopedics [113]. Metformin treatment for iMSCs cultured on CPC scaffolds dramatically enhances alkaline phosphatase activity, mineralizes nodule generation, and upregulates the expression of osteogenic markers, RUNX2 and osterix, which indicates that metformin has potential application in bone and periodontal regeneration in diabetic patients [114]. Anti-BMP2 antibody/BMP2 immune complex induces iMSCs to acquire osteogenic phenotype and mineralization, which suggests that antibody-induced osteogenic differentiation could be an alternative technology for iPSC-based tissue regeneration [115]. Kikuchi et al. recently differentiated iNCCs into iMSCs, which were differentiated into osteoblasts, adipocytes, and chondrocytes in vitro, and had capability to regenerate craniofacial bone in vivo without tumor formation [116].

3. Cell banking of iPSCs to improve efficiency

It is quite time-consuming to obtain personalized patient-derived iPSCs and amplify them to a large enough scale for clinical application. Considering the foreign human leucocyte antigens (HLA), the approach of genetic modification to generate a 'universal donor' or a cell bank consisting of HLA-typed iPSCs could reduce the immune rejection and also the dosage of immunosuppressive agents [117]. The HLA-typed iPSCs from healthy donors or the patients will benefit both allogeneic and autologous therapies. The process of banking iPSC cell lines that have homozygous HLA haplotypes is indicated as iPSC haplobanking [117]. The center for iPS Cell Research and Application, Kyoto University, is engaged in developing clinical grade iPSCs from HLA-homozygous donors [118]. Besides HLA-homozygous iPSCs, HLA-matched or histocompatible cell sources supply another strategy to construct iPSC stock. iPSCs have been generated successfully from dental pulp cells obtained from extracted third molars; three homozygous HLA loci (HLA-A, B, and DR) from 2 out of 107 donors were found, estimated to cover probably 20% of the Japanese population for best loci matches [119,120]. Fifty iPSC cell lines containing the same 3-HLA-loci could cover 90% of the Japanese population with perfect matches for these loci [121,122]. Xu et al. generated HLA-C-retained but HLA-A&B-disrupted immunocompatible iPSCs, 12 lines of which along with HLA-class II knockout were suggested to cover > 90% of the global population in terms of immunological compatibility [123]. The Korea National Stem Cell Bank has preserved 22 Good Manufacturing Practice-compliant homozygous HLA-type iPSC lines that cover HLA haplotype compatibility for 51% of the Korean population [124]. Eighteen kinds of quality tests including identity, sterility, consistency, stability, and safety had been performed to ensure the quality of the stocking cell lines. The European Bank for induced Pluripotent Stem Cells has deposited more than 900 iPSC lines [125]. There is still a long way to realize iPSC-based regenerative medicine products, thus it is indispensable to build a global network with comprehensive collaboration on tissue acquirement, iPSC reprogramming, in vitro passaging, quality control, data management, and ethical and legal framework.

4. Concluding remarks

The development of reprogramming technologies will facilitate iPSC generation without tumorigenicity and genetic instability. Additionally, the application of lineage-specific progenitor cells derived from iPSCs (e.g., iNCCs and iMSCs), cannot only restrict iPSC tumorigenicity, but also simplify procedures via feeder-free culture systems. Although iPSCs, iNCCs, and iMSCs have potential applications in periodontal and tooth regeneration, more comparative studies in vitro and in vivo and clinical trials need to be conducted. Given the structural complexity of teeth, it is crucial to elucidate the integrated mechanisms of all constitutive cells and the spatio-temporal interactions among them to generate functional teeth.

CRediT authorship contributions statement

Conceptualization: **Pan Gao**. Manuscript draft: **Pan Gao, Shan Liu, Makoto Ikeya**. Critical revision of the manuscript for important intellectual content: **Pan Gao, Xiaoyi Wang, Makoto Ikeya**.

Conflicts of interest

None

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