Chapter 6
Potential Use of Dental Stem Cells for Craniofacial Tissue Regeneration

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Abstract Mesenchymal stem cells such as bone marrow stromal cells and Adipose-derived stem cells are widely being used for clinical applications in regenerative medicine. Dental stem cell sources such as dental pulp stem cells, stem cells from human exfoliated deciduous teeth, periodontal ligament stem cells, stem cells from apical papilla, dental follicle progenitor cells, and tooth germ stem cells have also been started to be used for the same purposes. Since most dental-derived stem cells are of cranial neural crest origin, their use in the engineering of craniofacial structures holds promise in the near future. This chapter will discuss the potential applications of adult stem cells in craniofacial tissue engineering. Current knowledge about adult stem cells of dental and non-dental origin will be reviewed with respect to their regenerative capabilities and therapeutic potentials

Keywords Dental stem cells • Craniofacial tissue engineering • Differentiation

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Abbreviations

MSC	Mesenchymal stem cell
BMSC	Bone marrow stromal cell
ASC	Adipose-derived stem cell
DPSC	Dental pulp stem cell
SHED	Human exfoliated deciduous teeth
PDLSC	Periodontal ligament stem cell
SCAP	Stem cell from apical papilla
DFPC	Dental follicle precursor cell
TGSC	Tooth germ stem cell
hTGSC	Human tooth germ stem cell
TGF-β	Transforming growth factor-β
BMP	Bone morphogenetic proteins
PEGDA	Poly (ethylene glycol) diacrylate
PD	Population doubling rate
ALP	Alkaline phosphatase
BSP	Bone sialoprotein
DSP	Dentin sialoprotein
NeuN	Neuronal nuclear antigen
GAD	Glutamic acid decarboxylase
NFM	Neurofilament M
GFAP	Glial fibrillary acidic protein
CNPase	2,3-Cyclic nucleotide-3-phosphodiesterase
DMP 1	Dentin matrix protein-1
EMD	Enamel matrix derivatives
CAP	Cementum attachment protein
CP-23	Cementum protein-23

6.1 Introduction

In humans, the healing of craniofacial tissues frequently results in limited regeneration due to size and character of the defect. Functional replacement of such lost or damaged craniofacial tissues is one of the specific goals of tissue engineering [1, 2]. Recent developments in tissue engineering initiated new alternatives by utilizing biomaterials [3], gene therapy [4], signaling molecules [5] and stem cells [6] to regenerate craniofacial structures, aiming at the ideal of restitutio ad integrum. Until now, much has been learned about the single use of various biomaterials in the craniofacial region [7]. Various materials, such as natural or synthetic polymers [8, 9], ceramics, and composites [10], were used as tissue engineering scaffolds to promote cell migration and differentiation, extracellular matrix synthesis, and vascularization. Also, bioactive molecules were added to these scaffolds to enhance cell attachment,

new tissue formation, and angiogenesis [11]. However, none of these cell-free approaches were able to establish optimal tissue regeneration. Since mesenchymal stem cells (MSCs) play a pivotal role in the development of craniofacial structures, tissue engineering approaches using MSCs hold promise of providing a treatment for people suffering from craniofacial tissue and organ deficiencies [12, 13].

The craniofacial region involves various components, such as bone, nerves, connective tissue, glands, fat, teeth, and muscle. From this perspective, the reconstruction of these structures using stem cell-based approaches is a complex issue, but not impossible. Various attempts to date have been made to engineer the periodontium [14], cementum [15], temporomandibular joint [16], bone, [6] and fat tissue [17] using stem cells. Especially, MSCs derived from the bone marrow stroma (BMSCs) have been used extensively in craniofacial tissue engineering [18, 19]. Bone marrow-derived MSCs have the potential to differentiate into various lineages, and have therefore, been also clinically applied for treating different tissue disorders [20, 21]. Studies have shown that these multipotent adult stem cells are present in various tissues and organs, such as the nerve, skin, adipose, tendon, synovial membrane, and liver [22–26]. However, due to some reasons, such as diseases of bone marrow or surgical trauma during bone marrow isolation procedures, researchers are looking for alternative stem cell sources that require minimally invasive collection procedures.

Recent studies have revealed the presence of adult stem cells in tissues of dental origin as well [27]. Dental stem cells have the capability to undergo osteogenic, odontogenic, adipogenic, and neurogenic differentiation [28]. Since MSCs from dental tissue are obtained during regular orthodontic procedures, usage of that type of stem cell is easy, cost-effective, and does not raise additional safety and ethical concerns. Six different types of stem cells were isolated from dental tissues, such as dental pulp stem cells (DPSCs) [27], stem cells from exfoliated deciduous teeth (SHED) [29], periodontal ligament stem cells (PDLSCs) [30], stem cells from apical papilla (SCAP) [31], dental follicle precursor cells (DFPCs) [32] and tooth germ stem cells (TGSCs) [33]. Indeed, one important feature of these dental-derived cells is their ectomesenchymal origin, which makes them a good candidate for tooth regeneration studies [28].

6.2 Adult Stem Cells of Non-Dental Origin

Mesenchymal stem cells (MSCs) are populations of adult cells that reside in various tissues and organs, especially in the bone marrow, and maintain their regenerative potential through asymmetric mitotic cell division [18]. In other words, they have the ability to renew themselves, while differentiating into several specialized cell types of mesenchymal origin, termed as multipotency [34]. Upon need, tissue-specific MSCs have the genetic potential to repair or regenerate tissues from which they derive [12].

6.2.1 Bone Marrow-Derived Mesenchymal Stem Cells

Among various cell sources, Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) have been extensively studied for regenerating different types of tissues. These cells are frequently isolated from bone marrow aspirates from the iliac crest and live in close contact with the hematopoietic stem cells that have been successfully used in the treatment of leukemia for several decades. Under established culture conditions, BMSC is a heterogeneous cell population [35]. However, these mixed populations of BMSCs can be purified and homogenous groups can be immune selected using various surface markers [36].

Although no single marker to date has been shown to identify the MSCs, several markers have been reported to be typical for BMSCs. These markers include CD29, CD44, CD73, CD90, CD105, CD146, CD166, and STRO-1 as positive, CD11b, CD14, CD34, CD45, and HLA-DR as negative [35, 37–39]. According to the minimal criteria proposed by International Society for Cellular Therapy, human MSCs must at least express CD73, CD90, and CD105, and lack expression of CD14 or CD11b, CD79 alpha or CD19, CD34, CD45, and HLA-DR surface molecules [40].

BMSCs are plastic adherent and have the ability to produce colonies when seeded at very low cell densities, termed as clonogenicity [35]. Moreover, it has been shown that BMSCs are capable of differentiating, at least, into mesodermal cell lineages, such as bone, cartilage, tendon, adipose, and muscle [18]. Besides, several studies reported the transdifferentiation potential of BMSCs into cells of different germ layers, including neurons [41], hepatocytes [42], retinal cells [43] and myofibroblasts [44]. The plasticity of BMSCs is still controversial since it is not clear whether the expression of tissue-specific markers is caused by transdifferentiation or cell fusion of other bone marrow cells [45].

The use of BMSCs for promoting the biologic potential of scaffolds in craniofacial tissue engineering, especially the hard tissue regeneration, has gained interest within last 10 years. Stem cell delivery may be a particularly effective treatment alternative for craniofacial bone defects with an impaired healing. However, there is a need for optimal carrier materials that enable the delivery and maintenance of stem cells at the defect site. Various scaffold materials have been used in combination with BMSCs, including ceramics [46], calcium phosphates [47], synthetic polymers [48], composites [49] and titanium meshes [50] in vitro. Besides, animal studies (including rat, dog, pig, sheep species) mostly provided the evidence that the application of BMSCs in bony defects increased osteogenesis compared to untreated defects without MSCs [6, 51–54]. Recently, it has been shown that anatomically shaped human bone grafts can be engineered using BMSCs in controlled perfusion bioreactor systems [55].

However, translational research, involving human subjects, is more important for the establishment of a human craniofacial cell therapy protocol. The first pioneering study came from Warnke et al. 2004 [56]. They showed the repair of an extended mandibular discontinuity defect by growth of a custom bone transplant with bone marrow precursor cells inside the latissimus dorsi muscle of an adult male patient. Instead of

culture expanded cells, freshly isolated cells were used in this study and the patient related outcome was satisfying. In further studies, researchers also tried autologous stem cell transplantation for the treatment of maxillofacial defects in human subjects (Table 6.1). For a detailed understanding of bone regeneration using autologous stem cells, there are recent reviews on craniofacial bone tissue engineering [57–59].

Craniofacial structures also contain cartilage tissues in various regions, such as ear, nose, and temporomandibular joint. Since one direction of differentiation for BMSCs is the chondrogenic lineage, various attempts, mostly using 3D culture systems, have been made to establish cartilage regeneration in vitro [60–62]. The differentiation potential of BMSCs towards chondrocytes depends on supplementation with growth factors, mainly transforming growth factor-β (TGF-β) and bone morphogenetic proteins (BMPs) [63]. The in vitro regeneration of cartilage using BMSCs have been shown by utilizing different scaffold systems, growth factors and gene therapy [9, 64, 65]. There are also several reports on human subjects about the transplantation of BMSCs for cartilage repair [66, 67]. Besides, the clinical outcomes of BMSC implantation versus autologous chondrocyte implantation have recently been evaluated in a cohort study of 72 patients [68].

In recent years, it has been reported that mandibular condyle can be also engineered using BMSCs due to their osteogenic and chondrogenic differentiation ability [16]. BMSCs isolated from adult rats were induced in osteogenic and then chondrogenic culture medium, separately. Differentiated cells were photoencapsulated in a poly (ethylene glycol) diacrylate (PEGDA) hydrogel in two separate layers resembling the natural form of human mandibular condyle and then transplanted into immunocompromised mice. Histological results showed that the two stratified separate osteogenic and chondrogenic layers maintained their phenotypes after transplantation [16, 69]. Especially, the intercellular matrix of the chondrogenic layer exhibited a strong staining with cartilage related markers, such as safranin O and transplanted cells displayed characteristics of native chondrocytes.

6.2.2 Adipose-Derived Stem Cells

In recent years, Adipose-Derived Stem Cells (ASCs) have become an alternative multipotent cell source for use in craniofacial tissue engineering [13]. ASCs share some similarities with BMSCs by means of immunophenotype, differentiation potential, and clonogenicity [70, 71]. In vitro differentiation of ASCs into osteogenic, chondrogenic, adipogenic, and myogenic lineages have been confirmed in various studies [72, 73]. Especially, the osteogenic potential of ASCs has been intensively studied through the combination of various grafting materials both in vitro and in vivo [73–76]. Also, animal [74, 75] and human [77] studies utilizing ASCs have demonstrated the bone regenerative potential of these cells in different conditions. In a recent clinical study, Thesleff et al. 2011 [77] have successfully repaired large calvarial defects with the combination of beta-tricalcium phosphate graft material and autologous culture expanded ASCs in four patients.

Table 6.1 Human studies of craniofacial bone regeneration using MSCs

[ref.]	Patient numbers	Procedure	Cell source	Serum	Scaffold	Osteogenic medium	Growth factor	Cell number (cells/ml)
[78]	6	Sinus lifting/onlay plasty	hBMSCs	Xenogenic	β-TCP	+	PRP	1×10^7
[79]	14	Sinus lifting/onlay plasty	hBMSCs	Xenogenic	Thrombin/calcium chloride	+	PRP	1×10^7
[80]	6	Sinus lifting	hBMSCs	Autologous	β-TCP/HA	–	–	n/a
[81]	2	Alveolar cleft	hBMSCs	Autologous	DBM	–	–	2.5×10^6
[82]	1	Postextraction	hBMSCs -BRCs	Xenogenic	Gelatin sponge	–	–	1.5×10^7
[83]	23	Sinus lifting (osteotome)	hBMSCs	Autologous	Thrombin/calcium chloride	+	PRP	n/a
[77]	4	Cranioplasty	hASCs	Autologous	β-TCP	–	–	1.5×10^7
[84]	3	Alveolar cleft	hBMSCs	Autologous	β-TCP/HA	–	PDGF+PRF	$2{,}5\times10^6$

BRC bone repair cells, *TCP* tricalcium phosphate, *HA* hydroxyapatite, *DBM* demineralized bone matrix, *PRP* platelet rich plasma, *PDGF* platelet-derived growth factor, *PRF* platelet rich fibrin, *n/a* not available

Another potential application of ASCs is the reconstruction of soft tissues for facial cosmetic purposes due to their adipogenic properties. Although the number of published articles on this area is very few, ASC enriched fat grafts hold promise for the repair of mastectomy defects [85] and facial defects due to abnormalities, such as the progressive hemifacial atrophy [86]. Recently, several animal studies have suggested that ASCs could also be used for the repair of the facial nerve [87, 88]. Decellularized allogenic artery conduits seeded with ASCs were used for the reconstruction of transected facial nerves of rats and these tissue engineered constructs provided beneficial effects on functional facial nerve regeneration, but the findings were inferior to the nerve autografts [87].

In vitro differentiation of stem cells towards different lineages is usually performed with the use of various supplementations and growth factors. It is well established that both these exogenous factors [88] and the tissue environment [89] play a crucial role in the differentiation potential and extracellular matrix production of these cells. Recent knowledge also suggests that MSCs, either cultured in conditioned media [90] or co-cultured with other cell types [91], improve their differentiation ability towards the desired lineage. Although this evidence favors the use of non-cranial-derived MSCs (BMSCs, ASCs, etc.) in craniofacial tissue engineering [92], important differences exist between the characteristics and therapeutic potential of MSCs from different sources. BMSCs from iliac bone and alveolar bone have been shown to have different characteristics in terms of cellular activities. For example, iliac BMSCs formed more compact bone in vivo and were more responsive to osteogenic and adipogenic differentiation in vitro and in vivo, whereas alveolar BMSCs proliferated faster, expressed increased levels of ALP and deposited more calcium in vitro [93].

These data provide the evidence that the origin of MSCs must be taken into account when planning a differentiation route of MSCs for treating craniofacial discrepancies. Since the neural crest cells are thought to contribute to the development of most craniofacial tissues and organs, a regeneration protocol that utilizes stem cells of cranial neural crest origin might be more beneficial to achieve this goal.

6.3 Adult Stem Cells of Dental Origin

6.3.1 Stem Cells from Mature Dental Tissues

Although quite limited, human dental pulp has the ability to repair itself when either caries or trauma does not involve the pulp cavity [94]. This means that ectomesenchymal progenitor cells remain in the pulp tissue after the eruption of human teeth and are also responsible for the formation of new dentin. Previous studies reported that these progenitors can be induced to differentiate into odontoblast-like cells and are capable of producing dentin-like mineralized nodules [95, 96]. Using a human wisdom teeth model, the characterization of these heterogeneous populations of dental pulp stem cells (DPSCs) was first performed by Gronthos et al. 2000 [27].

Table 6.2 Immuno phenotyping of adult stem cells from different sources

	BMSCs	ASCs	DPSCs	SHED	PDLSCs	SCAP	DFPCs	TGSCs
CD3	–	–	–	–	–	n/a	–	n/a
CD9	+	+	+	n/a	+	n/a	+	n/a
CD10	+	+	+	+	+	n/a	+	n/a
CD13	+	+	+	+	+	+	+	n/a
CD14	–	–		–	–	–	–	–
CD29	+	+	+	+	+	+	+	+
CD31	–	–	–	–	–	n/a	–	n/a
CD33	–	–	–	–	–	n/a	–	n/a
CD34	–	–	–	–	–	–	–	–
CD44	+	+	+	+	+	+	+	+
CD45	–	–	–	–	–	–	–	–
CD56	–	–		+	n/a	n/a	+	n/a
CD59	+	+	+	n/a	+	n/a	+	n/a
CD73	+	+	+	+	+	+	+	+
CD90	+	+	+	+	+	+	+	+
CD105	+	+	+	+	+	+	+	+
CD106	+/–	+/–	+	+	+	+/–	+/–	n/a
CD117	–	–	–	–	–	–	–	n/a
CD133	–	–	–	n/a	n/a	n/a	+	–
CD146	+	+	+	+	+	+	+	n/a
CD166	+	+	+	+	+	+	+	+
STRO-1	+	+	+	+	+	+	+	+
SSEA-4	+	+	+	+	+	+	+	+
HLA-DR	–	–	–	–	–	–	–	–
OCT4	+	+	+	+	+	+	+	+
NANOG	+	+	+	+	+	+	+	+
Nestin	+	+	+	+	+	+	+	+
Sox2	+/–	+	+	+	+	+	+	+
Rex-1	+	+	+	+	+	+	+	n/a
ALP	+	+	+	+	+	+	+	+

+/– contradictory results in the literature, *n/a* not available

DPSCs have some similar characteristics with BMSCs such as high proliferation rate, colony-forming ability, differentiation potential under normal culture conditions [37] and also express several important mesenchymal markers, such as CD44, CD90, and CD105 (Table 6.2) [28]. Besides their dentinogenic potential, DPSCs have been reported to differentiate into osteogenic, chondrogenic, adipogenic, and myogenic lineages [97–99]. Recently, CD117 positive DPSCs have been reported to differentiate into high-purity hepatocyte-like cells [100].

Additionally, ecto-mesenchymal stem cells can also be isolated from the pulp of resorbing milk teeth, termed as stem cells from exfoliated deciduous teeth (SHED) [29]. When compared with DPSCs and BMSCs (Table 6.2), SHEDs are highly proliferative with an increased population doubling (PD) rate [101]. These cells have been shown to express STRO-1 and Oct-4, two important cell surface markers of

multipotent stem cells (Table 6.2) [102]. As seen in DPSC cultures, SHEDs express osteo/odontogenic cell markers, including alkaline phosphatase (ALP), bone sialoprotein (BSP), Cbfa1, and dentin sialoprotein (DSP) [29, 103]. SHEDs also express several neural markers, such as β III-tubulin, neuronal nuclear antigen (NeuN), glutamic acid decarboxylase (GAD), nestin, neurofilament M (NFM), glial fibrillary acidic protein (GFAP) and 2,3-cyclic nucleotide-3-phosphodiesterase (CNPase) [29]. In a previous study, SHED-derived neural-like spheres were transplanted into the striatum of parkinsonian rats and an improvement in the behavioral impairment was achieved [104]. Also, it has been recently reported that tooth-derived stem cells, SHEDs [105] and DPSCs [106], could be a useful tool for functional recovery after spinal cord injury. Adipogenic, myogenic, and chondrogenic differentiation have also been reported from SHED [107].

One treatment strategy in the craniofacial region using dental pulp-derived stem cells (DPSC and SHED) might be the regeneration of tooth structures, including pulp and dentin. When transplanted into immunocompromised mice, DPSCs displayed an ability to form dentin pulp-like complexes [108]. However, transplanted SHEDs were capable of establishing dentin pulp-like tissue [29]. Additionally, it has been shown that SHEDs have a higher capacity of osteogenic and adipogenic differentiation compared to DPSCs [101, 109]. Two recent studies demonstrated the osteogenic potential of SHED in critical size bone defects in pig mandibular [110] and mouse calvaria [111] in vivo. Using DPSCs, endodontic perforations were successfully repaired with a tissue engineering approach, involving dentin matrix protein 1 (DMP1) signaling molecule and a collagen scaffold, in immunocompromised mice [112]. Especially, the transplantation of $CD31^{-}/CD146^{-}$ side populations of DPSCs into an amputated in vivo pulp model resulted in complete pulp regeneration with vascular and neuronal compartments [113].

The periodontal ligament (PDL) is an interfacial connective tissue between alveolar bone and cementum, and contains progenitor cell populations that are responsible for the maintenance of the tooth in the alveolar socket against mastication forces. These progenitor cells have long been known to differentiate into cementoblasts and osteoblasts [114]. A previous study reported that these periodontal-derived stem cells display characteristics (osteogenic, adipogenic, and chondrogenic) similar to mesenchymal and other dental stem cells (Table 6.2), and termed them as periodontal ligament stem cells (PDLSCs) [30]. Especially, the expression of chondrogenic genes, early osteoblastic and adipogenic markers were enhanced in $STRO\text{-}1^{+}/CD146^{+}$ immunoselected PDLSC cultures [115]. Besides their osteogenic potential, PDLSCs express important markers for tendo/ligamentogenesis, including scleraxis and tenomodulin [116]. Moreover, a periodontium-like structure, including cementum and PDL, can be regenerated following transplantation of PDLSCs into immunocompromised mice [30, 117]. Several animal studies [118, 119] reported that autologous PDLSCs transplanted into surgically created periodontal defects were able to regenerate periodontal tissues and differentiate into functional osteoblasts and fibroblasts, thereby providing a treatment alternative for periodontitis.

Another treatment strategy using PDLSCs is the formation of a periodontal-like tissue around dental implants, in order to challenge the concept of osseointegration with biointegration. An organized periodontal tissue was found around titanium

implants seeded with PDLSCs and placed into maxillary molar sites of rats [120]. A similar approach involving human subject also revealed that new tissue with PDL characteristics, such as lamina dura and motility similar to teeth, was established at the bone implant interface [121]. Recently, it has been shown that heterogenous cultures of PDLSCs contain stem cells of neural crest origin, thus making them a useful tool in neuroregenerative and/or neurotrophic medicine [122].

6.3.2 Stem Cells from Immature Dental Tissues

During tooth development, ectomesenchyme-derived dental papilla cells are known to be responsible for root formation. While the root is being formed, dental papilla is entrapped by dentin that is produced by odontoblasts of dental lamina origin [123]. So, the dental pulp takes its final form and dental papilla protrudes more apically forming a cell rich zone at the apex. Previous studies have indicated that stem cells are also present in this apical part of dental papilla of the developing permanent teeth [31]. Therefore, these stem cells derived from the apical papilla (SCAP) can only be isolated from the apex of immature teeth at a certain development stage [124].

SCAP expresses several mesenchymal markers and lack hematopoietic markers similar to DPSCs and SHED (Table 6.2) [125]. Interestingly, SCAP expresses CD24 that is normally not present in DSPC and SHED cultures [28, 126]. Besides, when stimulated, these cells can undergo osteogenic and odontogenic differentiation in vitro [125]. Although the expression levels of osteo/dentinogenic markers in SCAP are lower than in DSPCs, SCAP have been reported to exhibit an increased proliferation rate, higher PD, better tissue regeneration capability, higher telomerase activity, and migration capacity in a scratch assay [127]. Additionally, ex vivo expanded SCAP was also found to differentiate into adipogenic and neurogenic lineages, as seen in DPSC and SHED [31]. A recent data suggested that canonical Wnt/β-catenin signaling favored the proliferation and odonto/osteogenic differentiation of SCAP [128]. Additionally, it has been reported that both SCAP and PDLSC could be used together in the regeneration of a root/periodontal complex capable of supporting a porcelain crown [127].

Dental follicle is a loose connective tissue and it surrounds the developing tooth (including enamel organ and dental papilla) before eruption. It is believed that DF is responsible for the establishment of periodontium, cementum, and alveolar bone until the tooth takes its final place [129]. This ectomesenchyme-derived sac-like tissue can be easily isolated during the extraction of impacted teeth. Recent evidence suggested that progenitor cells in the dental follicle (DFPCs) are plastic adherent and form clonogenic colonies similar to other dental stem cells when cultured in vitro [32]. DFPCs display fibroblastic morphology and express putative stem cell markers Notch-1 and Nestin [130]. Under specific culture conditions, DFPCs differentiated into osteogenic, neurogenic, and adipogenic lineages [32, 131]. When stimulated with enamel matrix derivatives (EMD) or BMP-2/-7, DFPCs expressed cementoblast markers, such as cementum attachment protein (CAP) and cementum protein 23 (CP-23) [132].

When supplemented with dexamethasone and/or insulin, human DFPSCs have been found to produce mineralized nodules in vitro. During osteogenic differentiation, the expression of some related genes (Osx, DLX-5, runx2, and MSX-2) remained unaffected [133]. However, the upregulation of DLX-3 as a response to osteogenic induction was found to influence the cell viability and osteogenic differentiation in DFPSC cultures [134]. Besides, bovine-derived DFPCs formed fibrous tissue surrounded by a mesothelium-like structure, but not cementum or bone, when transplanted into immunodeficient mice [32]. DFPCs are also capable of differentiating towards neurogenic lineage. After cultivation in serum replacement medium, containing culture supplement for glial cells, neurosphere-like cell clusters were established from DFPCs, and these cells were further differentiated into neuron-like cells by subculturing them on laminin and poly-L-ornithine substrates [135]. On the other hand, TGF-β was demonstrated to improve glial-like differentiation of DFPCs, but not neural like [136]. Recently, DFPC cell sheets were shown to have a better regeneration potential for periodontal tissues than PDLSC sheets, when subcutaneously transplanted into nude mice [137].

6.3.3 Tooth Germ Stem Cells

Until now, most studies cultured stem cells derived from immature tooth tissues in two portions by dissecting the dental follicle and apical papilla, separately. So, either DFPC or SCAP cultures were established. However, adult stem cells, that are responsible for tooth development, are derived from both ectoderm and the underlying mesenchyme. Therefore, reciprocal signaling pathways between these cell groups should be considered in designing a culture system from third molars [138]. The hypothesis of our studies was that the whole tooth germ should be used for preserving the stemness of the culture when isolating stem cells from immature third molars. Besides, the perfect dissection of the tooth germ tissue into dental follicle and apical papilla portions is impossible at the stage of early crown formation (unpublished data), thereby leaving some remnants from the adjacent tissue. Thus, in our cultures we have decided to isolate stem cells from the whole developing tooth organ, as done in the literature [139], and termed them as tooth germ-derived stem cells (TGSCs) (Fig. 6.1).

Human tooth germ tissues are derived from third molars and they are quite unique since embryonic tissues remain quiescent and undifferentiated until around age 6. Thus, human TGSCs are considered to be an ectomesenchymal source for isolating primitive pluripotent stem cells that could differentiate into multiple lineages. In our previous studies, we were able to isolate and characterize MSCs from human dental follicle (DFPCs) [140] and human tooth germ (hTGSCs) [33]. In the later study, we showed the differentiation of hTGSCs into osteogenic, adipogenic, and neurogenic cells, as well as tube-like structures in Matrigel assay [33]. Significant levels of sox2 and c-myc messenger RNA (mRNA) and a very high level of klf4 mRNA expressions were observed when compared with human embryonic stem cells. Recently, another group reported that stem cells derived from third molars of young donors

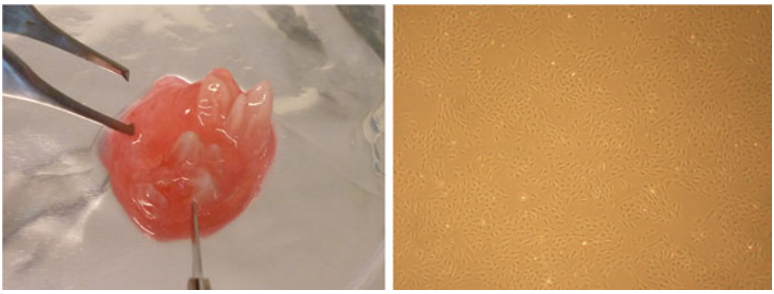

Fig. 6.1 Dissection of tooth germ tissue and morphology of TGSCs derived from pig (10× obj)

(10, 13, and 16 years old) could be reprogrammed to a pluripotent state (induced pluripotent stem (IPS) cells) by using retroviral vectors containing oct3/4, sox2, and Klf4 [141]. Expression of developmentally important transcription factors could render hTGSCs an attractive candidate for autologous transplantation since they can differentiate into various tissue types, such as osteoblasts, neurons, and vascular structures [33].

Interestingly, primary cultures of TGSCs readily express early neural stem cell markers, including nucleostemin, nestin, vimentin, and β-III tubulin [33]. Furthermore, the cryopreservation did not lead to a major change in the undifferentiated state of TGSCs [142]. According to the expression of neurogenic markers (β-III tubulin, nestin, and neuronal intermediate filament NFL), TGSCs also protect their neurogenic potential following long term cryopreservation [142], thereby making them a potential source for the treatment of neurodegenerative disorders. In a similar study [139], the neurogenic and hepatogenic characteristics of human tooth germ precursor cells (TGPCs) were evaluated. Especially, the transplantation of undifferentiated TGPCs into immunocompromised rats with experimentally established liver fibrosis led to improvement of liver function [139].

Although the number of published articles about TGSCs is extremely low, current findings provide important clues about the primitive characteristics of these cells. Thus, further studies, including transplantation protocols, needed to evaluate their regenerative potential in the craniofacial tissue engineering.

6.4 Conclusion

Stem cell sources have extensively been used for the treatment of craniofacial tissue defects since they have the capacity to originate a wide range of tissues. Generally, MSCs are preferred for such tissue regenerations. However, dental stem cells have also a self renewal and multilineage differentiation capacity. Besides, they are originated from cranial neural crest. Therefore, they have great potential to get used in craniofacial tissue engineering applications.

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