The similarity between human embryonic stem cell-derived epithelial cells and ameloblast-lineage cells

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This study aimed to compare epithelial cells derived from human embryonic stem cells (hESCs) to human ameloblast-lineage cells (ALCs), as a way to determine their potential use as a cell source for ameloblast regeneration. Induced by various concentrations of bone morphogenetic protein 4 (BMP4), retinoic acid (RA) and lithium chloride (LiCl) for 7 days, hESCs adopted cobble-stone epithelial phenotype (hESC-derived epithelial cells (ES-ECs)) and expressed cytokeratin 14. Compared with ALCs and oral epithelial cells (OE), ES-ECs expressed amelogenesis-associated genes similar to ALCs. ES-ECs were compared with human fetal skin epithelium, human fetal oral buccal mucosal epithelial cells and human ALCs for their expression pattern of cytokeratins as well. ALCs had relatively high expression levels of cytokeratin 76, which was also found to be upregulated in ES-ECs. Based on the present study, with the similarity of gene expression with ALCs, ES-ECs are a promising potential cell source for regeneration, which are not available in erupted human teeth for regeneration of enamel.

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**INTRODUCTION**

Regenerative medicine promises novel therapies for tissue regeneration, including the potential to replace missing teeth with bioengineered tissues. Tooth formation is guided by reciprocal signaling interactions between the cranial neural crest-derived mesenchymal cells (forming dentin and pulp) and ectoderm-derived dental epithelial cells (forming enamel). Classic tissue recombination experiments have been used to successfully regenerate mouse, rat and pig teeth 

Previous studies have shown that mouse embryonic stem cells and c-kit-positive mouse bone marrow cells can be differentiated into ameloblast-like cells. These studies of stem cells from mouse suggest that human embryonic stem cells (hESCs) could be a viable cell source for regeneration of dental epithelial cells. hESCs are derived from the inner cell mass of blastocyst-stage human embryos. hESCs can self-renew and give rise to all types of cell in the human body. When induced with bone morphogenetic protein 4 (BMP4) and α-retinoic acid (RA), hESCs differentiate toward cells with an epithelial morphology. Cytokeratin expression is a characteristic of epithelial cells, and the cytokeratin expression pattern of hESC-derived epithelial cells (ES-ECs) has not been characterized and compared with the cytokeratin expression patterns of other epithelia.

Cytokeratins (CKs) are the typical intermediate filament proteins of epithelia, which are known to be expressed in an epithelial type- and stage-specific manner. CKs are chemically very stable, long and unbranched filaments of ~10 nm in diameter, which are important for maintaining mechanical stability and integrity of epithelial cells and tissues at both the single cell and epithelial sheet levels. The CK gene family consists of 54 distinct functional genes in humans. CKs are heterodimers by pairing one type I keratin and one type II keratin (1:1) molecule.

In this study, we characterized the expression patterns of CKs and genes associated with early enamel organ epithelial differentiation in...
ES-ECs, as compared to human fetal skin epithelium (SE), fetal oral buccal mucosal epithelial cells (OEs) and ALCs. These results will direct our further studies to explore the potential for using ES-ECs to regenerate ALCs.

MATERIALS AND METHODS

Maintenance of hESCs

The hESC line (WA09) was purchased from the WiCell Research Institute (Madison, WI, USA). The cells were maintained on a layer of mitosin inactivated mouse embryonic fibroblasts (MEFs) in unconditioned medium: Dulbecco’s modified Eagle’s medium (DMEM)/Ham’s F-12 medium containing 20% Knockout serum replacement (Life Technologies, Carlsbad, CA, USA), non-essential amino acids (Millipore, Billerica, MA, USA), 1 mmol-L⁻¹ L-glutamine, 0.1 mmol-L⁻¹ betamercaptoethanol and 4 ng-mL⁻¹ basic fibroblast growth factor, 50 mg-mL⁻¹ penicillin and streptomycin (Sigma-Aldrich, St Louis, MO, USA).

Epithelial induction of hESCs

As described by Metallo and co-workers, BMP4 and +RA were used to induce differentiation of hESCs to an epithelial lineage. In brief, hESCs were grown for 2 days on Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) coated culture-ware in MEF-conditioned medium. The hESC line (WA09) was purchased from the WiCell Research Institute (Madison, WI, USA). The cells were maintained on a layer of mitosin inactivated mouse embryonic fibroblasts (MEFs) in unconditioned medium: Dulbecco’s modified Eagle’s medium (DMEM)/Ham’s F-12 medium containing 20% Knockout serum replacement (Life Technologies, Carlsbad, CA, USA) and RA (Sigma-Aldrich, St Louis, MO, USA). In parallel studies, lithium chloride (LiCl), a Wnt signaling activator, was added to the induction medium.

hESCs were induced for 7 days using the following conditions: Group A, 25 mg-mL⁻¹ BMP4 + 1 μmol-L⁻¹ RA; Group B, 25 mg-mL⁻¹ BMP4 + 1 μmol-L⁻¹ RA + 100 μmol-L⁻¹ LiCl; Group C, 25 mg-mL⁻¹ BMP4 + 1 μmol-L⁻¹ RA + 1 mmol-L⁻¹ LiCl; Group D, 12.5 mg-mL⁻¹ BMP4 + 1 μmol-L⁻¹ RA; Group E, 12.5 mg-mL⁻¹ BMP4 + 1 μmol-L⁻¹ RA + 100 μmol-L⁻¹ LiCl; Group F, 12.5 mg-mL⁻¹ BMP4 + 1 μmol-L⁻¹ RA + 1 mmol-L⁻¹ LiCl. Upon induction, cell morphology was recorded daily using a phase contrast microscope. After 7 days’ induction, cells were collected and total RNA was extracted using RNeasy Mini kit (Qiagen, Düsseldorf, Germany) according to manufacturer’s instruction. SuperScript III First-Strand Synthesis System (Life Technologies, Carlsbad, CA, USA) was used to synthesize cDNA. In brief, 2 μg total RNA, 200 ng random primers, 1 μL 10 mmol-L⁻¹ dNTP mix, 4 μL 5× first-strand buffer, 1 μL 0.1 mol-L⁻¹ DTT and 1 μL SuperScript III reverse transcriptase were used to constitute a 20-μL reaction, which was incubated at 50°C for 1 h.

Quantitative polymerase chain reaction (PCR) was carried out using the ABI 7500 system (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). Primers and TaqMan probes were used for detecting endogenous control 18S and target genes were purchased from Applied Biosystems. TaqMan probes can hybridize to the target DNA sequence and emit fluorescence as PCR reaction goes on. The intensity of released fluorescence is proportional to the amount of the PCR ampiclon. The real-time quantitative PCR (qPCR) conditions were as follows: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. To quantify the relative expression levels of the target gene, the comparative CT (threshold cycle) method was used. The following arithmetic formulas used are the following: \(\text{ACT} = \text{CT}_{\text{target}} - \text{CT}_{\text{GAPDH}}\) and \(\text{CT}_{\text{Linear}} = 2^{(-\Delta\text{CT}_{\text{condition1}} - \Delta\text{CT}_{\text{condition2}})}\). CT_{Linear} value represents the fold change in mRNA expression levels between the two conditions, assuming a doubling of the amplified product with each PCR cycle.

Characterization of amelogenesis-associated genes in differentiated cells

Conventional PCR was used to analyze the gene expression of Fgf8, a signaling molecule upregulated in placode stage of dental epithelial cells; Msx1, Msx2, Shh and Pitx2 have been identified to be important factors in early stages of amelogenesis. In addition, gene expression of amelogenin, the characteristic enamel matrix protein marker for secretory ameloblasts, was determined. The gene of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. A cDNA library of human fetal tooth organs was used as a positive control for amelogenin amplification. PCR was performed at 95°C for 5 min, 34 cycles of 95°C for 30 s, 57°C for 30 s and 72°C for 1 min, followed by 72°C for 5 min. Primer sequences for target genes are shown in Table 1. After amplification, equal volumes of PCR products were separated by electrophoresis on agarose gels.

Characterization of CKs in differentiated cells

All fetal tissues were collected from 16- to 20-week-old human fetal cadavers under guidelines approved by Committee on Human Research at the University of California, San Francisco. Human fetal OEs and ALCs were cultured following previously published protocols. Briefly, dissected fetal oral buccal mucosal epithelia were minced and further dispersed by incubating with 2 mg-mL⁻¹ collagene-disperase (Roche, Basel, Switzerland) at 37°C for 2 h. Dissected tooth organs were digested with 2 mg-mL⁻¹ collagene/disparase at 37°C for 2 h. After washing, the tissue mass was further digested with 0.05% trypsin/ethylene diaminetetraacetic acid (EDTA) for 5 min at 37°C. Epithelial cells were selectively grown in supplemented keratinoctye growth medium (KGM-2) (Lonza, Basel, Switzerland) with 0.05 mmol-L⁻¹ calcium, 1% penicillin and streptomycin on BD Primaria Tissue Culture Dishes (BD Biosciences, Franklin Lakes, NJ, USA).

Total RNA was purified from cultured OEs, ALCs and dissected fetal facial SE by using RNeasy Mini RNA kit (Qiagen, Düsseldorf, Germany). cDNA was synthesized by using SuperScript III First-Strand Synthesis System (Life Technologies, Carlsbad, CA, USA), and served as templates to amplify CKs using the same PCR conditions as described above. Primers used to amplify CKs were listed in Table 2.

| Genes   | Sequences of primers                             | Size/bp |
|---------|--------------------------------------------------|---------|
| Ang     | Forward GGCCTGACACCAAACTATOC                    | 383     |
|         | Reverse CCGCCTGACCTGCTGTCG                      |         |
| Fgf8    | Forward GTTGACTGCTGGCTCTCT                      | 220     |
|         | Reverse GAGTTGAGTGGCCAGATGA                     |         |
| Msx1    | Forward TCTCAGCCAGAAGATT                        | 342     |
|         | Reverse TCTCAGGCTCTGCTCTG                       |         |
| Shh     | Forward CCAATTACAAACCGGACATC                     | 339     |
|         | Reverse CCAGAATCCCATGCGAAGC                      |         |
| Msx2    | Forward ACACAGAGACATGCGAAGG                      | 222     |
|         | Reverse GCAGCATTTCAGCCTG                        |         |
| Pitx2   | Forward ACTTTACAGCCAGCAGCTC                      | 369     |
|         | Reverse GTGAGGAAACATGCTGT                       |         |
| GAPDH   | Forward ACCACGTCCATGCGATC                       | 452     |
|         | Reverse TCCACACCGTGTCTGT                        |         |
RESULTS

hESCs were induced toward an epithelial fate

hESCs were maintained on MEF feeder cells and formed typical tightly packed embryonic stem cell colonies (Figure 1a). Prior to induction, hESCs were passaged to Matrigel-coated culture ware, and still formed the tightly packed colonies (Figure 1b). After culture with epithelial induction medium for 7 days, hESCs transformed into a cobble-stone epithelial phenotype (Figure 1c), and some cells formed concentric cell nests indicated by arrows in Figure 1d. Expression of CK14, a CK marker for epithelial cells, was upregulated with the induction with BMP4 and RA (Figure 1e). Further increasing the concentration of BMP4 did not significantly enhance the efficiency of differentiation. Additional LiCl was capable of inducing amelogenin associated genes ES-ECs showed upregulation of early stage of odontogenesis-markers including BMP4, RA and LiCl was capable of inducing amelogenin significantly enhance the efficiency of differentiation. Additional LiCl was capable of inducing amelogenin associated genes.

ES-ECs showed upregulation of early stage of odontogenesis-associated genes Fgf8 and Msx1

As shown in Figure 2, differentiated ES-ECs (lane A–C) expressed Fgf8 and Msx1 in addition to Shh, Msx2 and Pitx2 that were expressed by non-induced hESCs (lane F). ALCs (lane E) and OEs (lane D) expressed Msx1, Msx2 and Pitx2, but no Fgf8 and Shh. None of inducers including BMP4, RA and LiCl was capable of inducing amelogenin expression in those induced epithelial cells. Although there was no detectable expression of Fgf8 and Msx1 in hESCs (lane F), ES-ECs (lane A–C) expressed detectable Msx1, similar to that of ALCS (lane E). We did not find any expression of Shh in ALCS at mRNA level, though mRNA expression of this molecule was detected in all the induced ES-ECs. Pitx2 and Msx2 were detected in all groups (lane A–F). Gene expression of secretory ameloblast specific enamel matrix protein amelogenin was not detected in any of the cultured cells (lane A–F). cDNA library of human fetal tooth bud was used as a positive control for amelogenin expression. GAPDH used as an endogenous control was detected in all groups with the similar intensity.

Both ES-ECs and ALCS expressed CK76

The pattern of CK expression is shown in Figure 3a. CK71, CK5, CK14, CK7, CK19, CK13, CK2, CK1, CK10, CK6 and CK16 were the CKs that

Table 2 Sequences of primers used to patterning cytokeratins

| Genes | Forward | Reverse | Size/bp |
|-------|---------|---------|--------|
| Krt2  | TTAGTGGCTGGAGGAGGT | GCTGCTGATATACCCCTGGA | 485 |
| Krt76 | CAGTTCACCTCCTCCAGCTC | GCACCTTGTCCAGGTAGGAG | 400 |
| Krt1  | AGGAGGTGGACGTGGTAGTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt10 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt16 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt25 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt39 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt42 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt43 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt44 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt45 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt46 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt47 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt48 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt49 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt50 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt51 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt52 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt53 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt54 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt55 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt56 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt57 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt58 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt59 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt60 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt61 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt62 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt63 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt64 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt65 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt66 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt67 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt68 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt69 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt70 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt71 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt72 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt73 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt74 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |
| Krt75 | AGGAGGCAAATTGGTTGTTG | AGGAGGCAAATTGGTTGTTG | 484 |

Figure 1 hESCs adopted an epithelial phenotype after induction. (a) hESCs grown on mouse embryonic fibroblast feeder layer formed well-defined embryonic stem cell colonies containing epithelialoid cells on the periphery and polygonal cells within the colony. (b) After being transferred to a feeder-free culture-ware, hESCs on Matrigel grew as monolayer. Colonies were well formed. Monolayer with 12.5 ng/mL BMP4 and 1 µmol/L RA for 7 days, hESCs adopted a cobble-stone like morphology. (c) A representative phase contrast microscopy image is shown of hESCs induced with 12.5 ng/mL BMP4 and 1 µmol/L RA and formed concentric cell nests indicated by red arrows. (d) CK14 expression was significantly upregulated upon the induction. Induction with 12.5 ng/mL BMP4 and 1 µmol/L RA resulted in an average 44-fold upregulation of CK14 as compared to that of control hESCs (column D). The columns represent mean values plus standard deviation of three independent experiments. Further increasing the concentration of BMP4 did not significantly enhance the efficiency of differentiation. Additional LiCl did not have any synergistic effects on upregulation of CK14 in the induced cells.

Characterization of hESC-derived epithelial cells

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Figure 3 Conventional PCR analysis shows the expression pattern of CKs in human ALCs, fetal OEs, fetal SE and ES-ECs. (a) CK25 was only present in SE while absent in ALCs, OEs and ES-ECs. CK4 was present in both ALCs and OEs, while absent in SE and ES-ECs. (b) One representative DNA electrophoresis image shows that CK76 was present in ALCs, ES-ECs and OEs, while absent in SE and hESCs. ALC, ameloblast-lineage cell; BMP4, bone morphogenetic protein 4; CK, cytokeratin; ES-EC, hESC-derived epithelial cell; hESC, human embryonic stem cell; OE, oral epithelial cell; SE, skin epithelium.
could be detected at mRNA level in all ALCs, OEs, SE and ES-ECs. CK25 was only detected in SE, and CK4 was mainly detected in ALCs and OEs. Expression of CK76 was more intense in ALCs, and was somewhat less intense in ES-ECs and OEs, but was not detected in SE and hESCs (Figure 3b).

**DISCUSSION**

The focus of this study was to determine whether the epithelial cells derived from hESCs could be used as a source of epithelial progenitor cells for ameloblasts. In this study, we demonstrated that BMP4 at 12.5 ng·mL⁻¹ and RA at 1 μmol·L⁻¹ are sufficient to differentiate hESCs into cells with an epithelial morphology and upregulated CK14. Increased concentrations of BMP4 to 25 ng·mL⁻¹ did not further enhance the efficiency of induction. BMP4 has been characterized as an inducer of epidermal differentiation.22 The effect of RA on epithelial lineage specification depends on the context of bone morphogenetic protein signaling.15

We conducted further studies to differentiate ES-ECs towards amelogenin-producing cells by using LiCl. LiCl is known to be an activator of Wnt signaling.23 Wnt signaling plays a critical role during tooth development,24–29 as shown by the lack of Lef1, a downstream effector of Wnt signaling pathway, which results in developmental defects in all epithelial appendages including tooth.30 However, supplementation of LiCl in the inducing medium did not result in the expression of amelogenin in ES-ECs.

The cultured ALCs and ES-ECs did not have any detectable amelogenin expression, indicating that neither cell type had reached a stage of differentiation characteristics of secretory ameloblasts. Conditioned medium collected from human ALCs could not induce ES-ECs to express amelogenin. We have found that coculture of ALCs with either Matrigel or dental pulp stem cells can result in upregulated amelogenin expression.31 Therefore, further differentiation of ES-ECs to secretory ameloblasts likely requires a complex of signaling molecules derived from dental mesenchyme or extracellular matrix.

In addition to Msx2 and Pitx2, ES-ECs expressed odontogenic associated genes including Fgf8, Msx1 and Shh. Fgf8 and Msx1 have been identified to be critical for the initial stage of tooth formation. Transgenic mice carrying a homozygous Msx1 germline mutation display a phenotype of complete absence of the incisor.32 Inactivation of Fgf8 can severely disturb mandibular and maxillary development with no molar teeth formed, while the incisor remains.33 Expression of Fgf8 and Msx1 in the ES-ECs is a promising finding toward identifying a cell source for ameloblast regeneration. Shh is either temporally expressed early at the epithelial thickening stage or later at the enamel knot of tooth formation. Shh did not present in human ALCs, which are derived from bell stage of tooth organs. In contrast, ES-ECs do express Shh, which further supports the possibility that ES-ECs are similar to early differentiating enamel organ epithelial cells.

We sought to characterize these cells utilizing Cks as markers specific for different types of epithelial cells. For example, CK25 is among the group of Cks that are highly specific for the inner root sheath (IRS) of the hair follicle.34 The keratinocytess of all three IRS compartments including the Henle layer, the Huxley layer and the IRS cuticle35–36 synthesize CK25.37 Our studies indicated that CK25 was certainly specific to human fetal skin epithelia, which contain hair follicles, while no CK25 expression was detected in hESCs, human fetal OEs, ALCs and ES-ECs, although skin epithelia, mucosal epithelia and ALCs are all derived from ectoderm.

The paired type II keratin CK4 and the type I keratin CK13 indicates a mucosal path of differentiation for the non-keratinizing internal stratified squamous epithelia. A previous study reported that CK4 is present in the entire suprabasal compartment of mucosal stratified squamous epithelia, while it is completely absent in the epidermis.38 In this study, expression of CK4 in both human oral buccal mucosal epithelial cells and ALCs which originate from the local thickened oral epithelial cells,39 suggests the same origin of these two epithelial sources. No CK4 was detected in human fetal facial SE, which confirmed that this CK is specific for mucosa and non-keratinized epithelia.

It is interesting that except for SE, all ALCs, OEs and ES-ECs expressed CK76, which is known to be expressed in suprabasal cells of oral masticatory epithelia. Given the fact that OEs and ALCs are derived from the same progenitor cell source, the upregulation of CK76 in ES-ECs suggests that ES-ECs have increased homology to ALCs and OEs, but do not commit to the fate of SE. Combined with the fact that ES-ECs expressed Fgf8, Msx1 and Shh, we anticipate that ES-ECs hold the potential to be differentiated into an ameloblast phenotype.

**CONCLUSION**

In this study, we have identified the effective concentrations of BMP4 and RA to drive differentiation of hESCs toward an epithelial lineage phenotype. We found that this protocol could increase the expression of Fgf8, Shh and Msx1 in ES-ECs. Expression of Msx1 in ES-ECs was similar to the levels found in ALCs. ES-ECs had upregulated expression of CK76, a CK that is differentially expressed in ALCs as compared to SE. These studies suggest that these ES-ECs are useful as an alternative cell source to regenerate enamel-forming cells as availability of dental epithelial cells in humans is significantly constrained. Studies to identify factors including the human dental mesenchyme that can promote the further differentiation of ES-ECs will further facilitate the use of hESCs and ES-ECs for tooth tissue regeneration.

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