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Integration-Free Reprogramming of Lamina Propria Progenitor Cells

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Abstract

Producing induced pluripotent stem cells (iPSCs) from human tissue for use in personalized medicine strategies or therapeutic testing is at the forefront of medicine. Therefore, identifying a source of cells to reprogram that is easily accessible via a simple noninvasive procedure is of great clinical importance. Reprogramming these cells to iPSCs through nonintegrating methods for genetic manipulation is paramount for regenerative purposes. Here, we demonstrate reprogramming of oral mucosal lamina propria progenitor cells from patients undergoing routine dental treatment. Reprogramming was performed utilizing nonintegrating plasmids containing all 6 pluripotency genes (*OCT4, SOX2, KLF4, NANOG, LIN28*, and *cMYC*). Resulting iPSCs lacked genetic integration of the vector genes and had the ability to differentiate down mesoderm, ectoderm, and endoderm lineages, demonstrating pluripotency. In conclusion, oral mucosal lamina propria progenitor cells represent a source of cells that can be obtained with minimal invasion, as they can be taken concurrently with routine treatments. The resulting integration-free iPSCs therefore have great potential for use in personalized medicine strategies.

Keywords: oral mucosa, human induced pluripotent stem cells, IPS cells, hiPSCs, stem cells, regenerative medicine

Introduction

The oral mucosal lamina propria (OMLP) harbors a population of multipotent and potently immunosuppressive progenitor cells (PCs; Davies et al. 2010; Davies et al. 2012). Given the well-reported scarless wound-healing ability of the oral mucosa, OMLP-PCs represent a readily accessible cell source with favorable growth kinetics and potential utility for a variety of personalized regenerative medicine applications.

In the late 1950s, reprogramming cell fate was achieved via nuclear transplantation to redirect cell phenotype (Gurdon et al. 1958), and in 2006 Takahashi and Yamanaka built on this work. They reported the identification of transcription factors that could reprogram adult somatic tissue from mouse dermal fibroblasts to an embryonic stem cell–like state. This process was termed *induced pluripotency*.

These induced pluripotent stem cells (iPSCs) were produced via viral introduction of 4 transcription factors (*OCT4, SOX2, KLF4*, and *cMYC*). This work has stimulated research into many disparate disease states given the potential use of iPSCs as a personalized cell source for cellular replacement strategies and to provide more accurate disease modeling. This is of particular importance for cell types with limited clinical sources (e.g., heart; Takahashi and Yamanaka 2006; Takahashi et al. 2007).

Several cell types from the oral cavity have successfully been reprogrammed utilizing genome integration of pluripotency genes—including gingival fibroblasts (Eugusa et al. 2010; Wada et al. 2011), oral mucosal fibroblasts (Miyoshi et al. 2010), and stem cells from the dental pulp, apical papillary, and human deciduous teeth (Yan et al. 2010).

Generation of iPSCs through nongenome integrating reprogramming strategies is paramount for safer regenerative medicine applications. This has been achieved via a variety of approaches, including nonintegrating adenoviral vectors (Stadtfeld et al. 2008), delivery of factors via PiggyBac transposition (Woltjen et al. 2009), episomal vectors incorporating the Epstein-Barr virus OriP and EBNA sequences (Yu et al. 2009), vectors based on the Sendai virus (Fusaki et al. 2009), and direct reprogramming through transient mRNA (Warren et al. 2010). An approach based on a lentiviral stem cell cassette with the cre/loxP system to excise the cassette following reprogramming has been described (Somers et al. 2010) and utilized for the reprogramming of apical papilla stem cells (Zou et al. 2012).

Given the accessible nature of OMLP-PCs, their favorable growth kinetics, and scarless wound-healing properties of the oral mucosa, we sought to determine the feasibility of whether

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OMLP-PCs could be reprogrammed through nonintegrating plasmid vectors to an induced pluripotent state as a platform for future studies assessing the clinically utility of such a cell population for regenerative medicine strategies.

Materials and Methods

Cell Culture

Oral cells were isolated from 6-mm biopsies of healthy oral (buccal) mucosa from 3 patients at the School of Dentistry, Cardiff University, following ethical approval (09/WSE03/18) and informed patient consent as previously reported (Stephens et al. 1996). OMLP-PCs were then isolated by differential adhesion to fibronectin as previously described (Davies et al. 2010). H9 human embryonic stem cell (hESC) lines from WiCell were utilized as positive control and cultured as previously described (Thomson et al. 1998). Later, established OMLP-iPSC colonies were passaged enzymatically with 1 mg/mL of collagenase and seeded onto fresh tissue culture plates prepared with inactivated mouse embryonic fibroblasts (iMEFs; Conner 2001) at a density of 1.4×10^4 cells/cm^2. These cells were obtained from animals sacrificed by a qualified technician under schedule 1 of the UK Animals Scientific Procedures Act of 1986. All cells were cultured in a humidified incubator at 37 °C and 5% CO_2.

Nucleofection

Ten-centimeter tissue culture plates were preseeded with 8×10^5 iMEFs and incubated for 24 h in mouse embryonic fibroblast medium: Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2mM L-glutamine, 100 U/mL of penicillin, 100 µg/mL of streptomycin sulphate, and 0.1mM nonessential amino acids (all from Invitrogen). Mouse embryonic fibroblast medium was removed and replaced with foreskin fibroblast medium—HFF containing MEM (Eagle; Invitrogen) supplemented with 10% fetal bovine serum, 2.0mM GlutaMAX (Invitrogen), 0.1mM β-mercaptoethanol (Sigma-Aldrich), 100 U/mL of penicillin, 100 µg/mL of streptomycin sulphate, and 0.1mM nonessential amino acids (Yu et al. 2009). Then 1×10^6 OMLP-PCs between population doubling levels 15 and 20 were pelleted and resuspended in 100 µL of nucleofector solution (NHDF-VPD-1001; Lonza). Plasmid DNA from 2 plasmids—7.3 µg of pEP4 E02S CK2M EN2L containing *OCT4, SOX2, NANOG, KLF4, LIN28*, and *cMYC* gene inserts and 3.2 µg of pEP4 E02S ET2K containing *OCT4, SOX2, SV40LT*, and *KLF4* gene inserts (pEP4 E02S CK2M EN2L and pEP4 E02S ET2K were a gift from James Thomson; Addgene plasmids 20924 and 20927, respectively)—were added, mixed, and nucleofected with program U-20 of the Amaxa. Following transfection, cells were immediately seeded onto iMEF plates. A transfection control was set up with an eGFP plasmid to calculate transfection efficiency. On day 3 posttransfection, medium was replaced with fresh hESC medium consisting of DMEM/F-12 1:1 supplemented with 20% knockout serum replacement (Invitrogen), 0.1mM nonessential amino acids, 1mM L-glutamine, 0.1mM β-mercaptoethanol, and 100 ng/mL of basic fibroblast growth factor (bFGF; Peprotech). Medium was changed daily and switched to mouse embryonic fibroblast–conditioned medium, prepared from iMEFs seeded into a 6-well plate at a density of 5×10^4 cells/cm^2 in 2 mL/well of mouse embryonic fibroblast medium. After 24 h, the feeder layers were washed twice with 2 mL/well of phosphate-buffered saline (PBS), and this was replaced with 2 mL/well of hESC medium without bFGF. Medium was collected and replaced with fresh hESC medium daily from day 8 posttransfection until cell colonies began to form on days 18 to 25. Potential iPSC colonies were identified through a live alkaline phosphatase staining kit (Molecular Probes). Colonies were picked, expanded, and characterized as previously described (Yu et al. 2009).

Reverse Transcription Polymerase Chain Reaction for Pluripotency Markers

Total RNA was extracted from OMLP-PCs, H9, and OMLP-iPSCs via the traditional Trizol/chloroform method; 0.5 µg of RNA was reverse transcribed with M-MLV reverse transcriptase per the manufacturer's protocol (Promega). A master mix of 12 µL of Platinum Blue Super Mix (Invitrogen) was mixed with 0.5 µL of 1µM forward and reverse primers specific to the gene of interest (*βACTIN, OCT4, SOX2, LIN28, KLF4, NANOG*, and *cMYC*; sequences and annealing temperatures in Appendix Table). One microliter of sample cDNA was added. Amplification was performed via the following program: initial denaturation for 10 min at 95 °C, 40 cycles of 95 °C for 5 min, 30-s annealing step at the optimal temperature for each primer, and elongation at 74 °C for 1 min, finishing with a final elongation at 74 °C for 10 min. Products were viewed following electrophoresis on standard TAE agarose gels. All primer products were sequenced by Central Biotechnology Services with a 16-capillary genetic analyzer (3130xl; Applied Biosystems).

Polymerase Chain Reaction Analysis of Genomic and Episomal DNA

Episomal DNA was extracted from OMLP-iPSCs between passages 9 and 14 via a QIAprep Kit, and Genomic DNA was extracted with the QIAamp DNA Mini Kit per the manufacturer's instruction (Qiagen). DNA samples were amplified by utilizing Taq polymerase per the manufacturer's protocol (Promega). A master mix was combined with 0.5 µL of 1µM forward and reverse primer for genes of interest, designed to incorporate sections of the plasmid to check for presence of plasmid in genomic DNA isolated from OMLP-iPSCs as well as endogenous *OCT4*. Extracted episomal and genomic DNA of OMLP-PCs was used as a negative control (sequences and annealing temperatures in Appendix Table; Yu et al. 2009).

Quantitative Polymerase Chain Reaction

cDNA from OMLP-PC samples of 3 patients was used to establish the level of pluripotency gene expression relative to that seen in hESCs (H9). Taqman Gene Expression Assays for

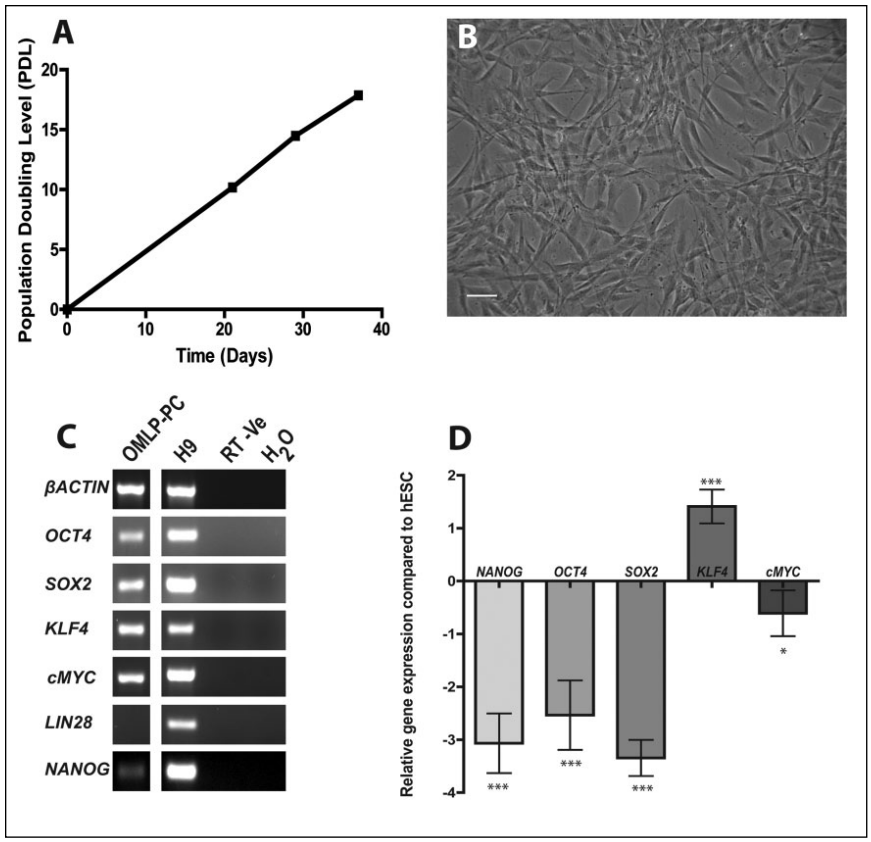

Figure 1. Characterization of oral mucosal lamina propria progenitor cells (OMLP-PCs) prior to reprogramming. (**A**) Population doubling levels for OMLP-PCs illustrating the point at which the cells were reprogrammed (levels 15 to 20). (**B**) Bipolar morphology of OMLP-PCs prior to reprogramming (scale bar = 100 µm). (**C**) End-point reverse transcription polymerase chain reaction (PCR) demonstrating expression of *OCT4, SOX2, NANOG, KLF4*, and *cMYC* in OMLP-PCs cells. No template controls for reverse transcription (RT-ve) and PCR reaction (H_2O). (**D**) Quantitative PCR demonstrating expression levels of pluripotency markers relative to H9 cells (n = 3; ±SD). *P < 0.05. ***P < 0.001.

OCT4, SOX2, NANOG, KLF4, and *cMYC* were performed per the manufacturer's protocol (Invitrogen). *GAPDH* was used as a reference gene. A standard curve ensured linear reactions of the target genes of interest. H9 cDNA was used as a standard, and 10-fold serial dilutions were performed. Each sample was assessed in triplicate. Reaction conditions included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles, a denaturation step at 95 °C for 15 s, then an annealing step at 60 ºC for 30 s. The $2^{-\Delta\Delta Ct}$ method was adopted to analyze the relative quantities of gene expression (Livak and Schmittgen 2001). Statistical analysis was performed via a t test on GraphPad InStat software (GraphPad Software, Inc.), and statistical significance was assumed when $P < 0.05$.

Differentiation

Passaged OMLP-iPSC colonies were seeded in 10-cm-diameter bacteriological-grade petri dishes in hESC medium without bFGF. Cells were refed every second day. Embryoid bodies (EBs) developed over a period of 8 d before differentiation was initiated. Chamber slides were precoated with 100 µL of Matrigel at a concentration of 0.1% (v/v) in hESC medium without bFGF for 1 h at 37 ºC / 5% CO_2. Plates were removed from the incubator and left at room temperature overnight. Three media conditions were used to initiate differentiation: 1) hESC medium alone, 2) hESC medium supplemented with 10µM retinoic acid (diluted in dimethyl sulfoxide; both Sigma-Aldrich), and 3) hESC medium supplemented with the dimethyl sulfoxide diluent as a vehicle control. Stock EBs were collected, pelleted, and resuspended in each of the 3 media conditions before seeding 250 µL onto the Matrigel-coated chamber slides. Medium was exchanged every other day for 14 d.

Immunocytochemistry

OMLP-PCs, H9, OMLP-iPSCs, and differentiated OMLP-iPSCs were fixed in 4% (v/v) paraformaldehyde for 15 min at room temperature. Cells were permeabilized with 0.1% (v/v) Triton X-100 for 20 min. Nonspecific binding of the secondary antibody was blocked with a solution of 2% (w/v) bovine serum albumin (BSA; diluted in PBS) for 1 h. Primary antibody—rabbit polyclonal antibody against BRACHYURY (ab20680, 5 µg/mL), OCT4 (ab19857, 2.8 µg/mL), mouse monoclonal antibodies against α-1-FETOPROTEIN (ab3980, 5 µg/mL), βIII-TUBULIN (ab7751, 2 µg/mL), SOX2 (ab75485, 1.25 µg/mL), SSEA-4 (ab16287-200, 4.2 µg/mL), TRA-1-60 (ab15830-100, 4 µg/mL), and TRA-1-81 (ab16289, 4 µg/mL; all from Abcam)—and a mouse monoclonal antibody against SSEA-5 (2.5 µg/mL; a gift from Dr. Micha Drukker, Stanford University) were all diluted in 2% (w/v) BSA in PBS and incubated in contact with the cells at 4 °C overnight. The chamber slides were then washed 3 × 5 min with PBS, and the secondary antibodies—swine anti-rabbit (F0205, 0.81 g/L) and rabbit anti-mouse (F0261, 2.3g/L; both Dako)—were diluted 1:50 in 2% (w/v) BSA in PBS and incubated in contact with the cells for 1 h at room temperature. Chamber slides were then washed 3 × 5 min with PBS before the nuclei were counterstained with DAPI-containing (1.5 µg/mL) mounting medium (Vector Shield). Appropriate IgG and IgM controls (Santa Cruz Biotechnology) were utilized to verify specificity by absence of staining.

Results

Oral Mucosa Lamina Propria PCs Constitutively Express Low Levels of Pluripotency Markers

OMLP-PCs were isolated, cultured, and utilized for reprogramming at a population doubling level of 15 to 20 (Fig. 1A). Cells demonstrated a typical fibroblast-like morphology prior to reprogramming (Fig. 1B). The pluripotency markers *OCT4, SOX2, NANOG, KLF4, cMYC*, and *LIN28* were analyzed, as these are the classical iPSC factors utilized to reprogram cells back to their pluripotent state (Takahashi et al. 2007; Yu et al. 2007). H9 hESCs provided a positive control. OMLP-PCs demonstrated expression of all markers with the exception of *LIN28* (Fig. 1C). To quantitate the relative level of expression of these genes, quantitative polymerase chain reaction was undertaken. H9 hESCs were utilized as the calibrator, as they expressed all the pluripotency genes of interest. Compared with hESCs, expression of *OCT4, SOX2, NANOG*, and *cMYC* was found to be significantly (*SOX2*, $P < 0.0001$; *OCT4* and *NANOG*, $P < 0.001$; *cMYC*, $P < 0.05$) lower in OMLP-PCs from 3 patients (Fig. 1D). Expression of *KLF4* was the only pluripotency gene expressed at a significantly higher level in OMLP-PC samples when compared with hESCs (*KLF4*, $P < 0.001$).

Oral Mucosa Lamina Propria Progenitors Are Positive for hESC-Like Markers after Nonviral Integrating Reprogramming

The low-level pluripotency gene expression led us to utilize plasmids containing all 6 pluripotency genes (*OCT4, SOX2, NANOG, KLF4, LIN28*, and *cMYC*) to attempt to reprogram the OMLP-PCs without the need for viral integration of the sequences. Transfection efficiency of the control eGFP plasmid in OMLP-PCs was found to be 63.6% (SD, 2.771%; Fig. 2B). Live alkaline phosphatase staining confirmed the presence of potential iPSC colonies on day 20 (Fig. 2C), as reported for iPSCs derived from other cells types (Fusaki et al. 2009; Lu et al. 2011; Merling et al. 2013). Following nucleofection and culture of the OMLP-PCs, putative iPSC colonies were clearly discernible that were similar in appearance to hESCs—namely, with tight colony boundaries and cells with a high nuclear:cytoplasmic ratio (Fig. 2D).

hESC-like colonies were expanded in culture, first by mechanical means and then by enzymatic passaging. Immunocytochemical analysis was then undertaken for typical hESC markers, including 2 transcription factors (OCT4 and SOX2) and 4 cell surface markers (SSEA-4, SSEA-5, TRA-1-60, and TRA-1-81). H9 cells were cultured as previously described and used as a positive control for the markers (see Appendix Fig.). OMLP-PCs that had not been nucleofected with the plasmids were used as a comparative control to ascertain the presence or absence of staining prior to reprogramming (see Appendix Fig.). The hESC transcription factors OCT4 and SOX2 and cell surface markers SSEA-4, SSEA-5, TRA-1-60, and TRA-1-81 were found to be present in all ($n = 6$) OMLP-iPSC-like

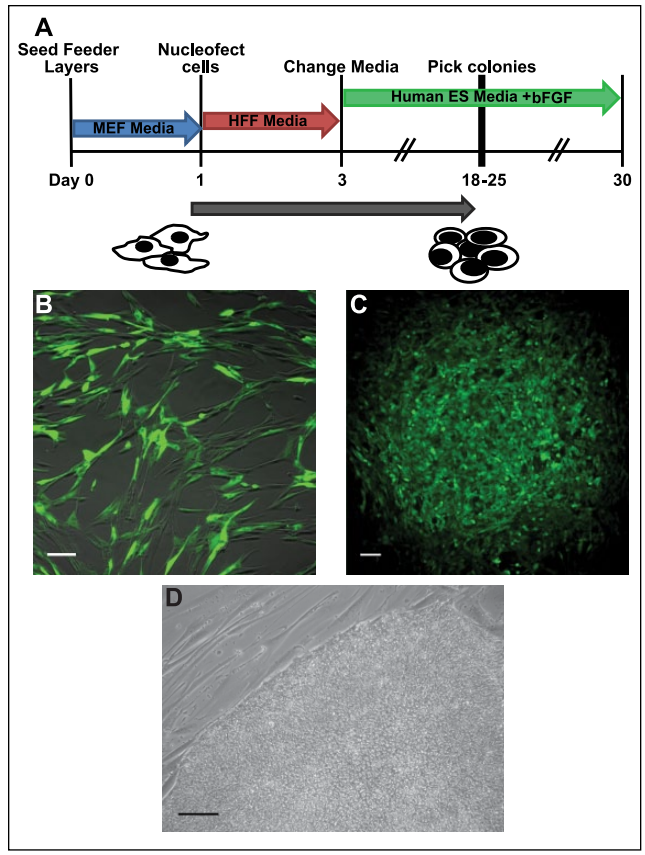

Figure 2. Reprogramming of oral mucosal lamina propria progenitor cells (OMLP-PCs). (**A**) Steps followed for reprogramming of OMLP-PCs. (**B**) Phase/GFP image demonstrating transfection of OMLP-PCs with eGFP plasmid to establish transfection efficiency. (**C**) Live alkaline phosphatase staining of developing induced pluripotent stem cell colonies on day 20. (**D**) Phase image of an example induced pluripotent stem cell colony with tight colony boundary and cells with high nuclear:cytoplasmic ratio. Scale bars = 100 µm. bFGF, basic fibroblast growth factor; ES, embryonic stem cell medium; HFF, foreskin fibroblast medium; MEF, mouse embryonic fibroblast.

cultures (Fig. 3A–F), suggesting that reprogramming was most likely to have occurred. Primary omission control, swine anti-rabbit secondary omission control, and IgM and IgG (rabbit) controls were negative. Importantly, polymerase chain reaction confirmed that there was no vector sequence present in the genomic DNA (G) isolated from the OMLP-iPSCs after cell reprogramming and colony expansion, as demonstrated by absence of bands in the iPSC 1G, 14G, and 40G lanes (Fig. 3G). Residual vector sequence was present in only the episomal DNA (E) isolated from the OMLP-iPSCs, as demonstrated by presence of bands in iPSC 14E and 40E (Fig. 3G).

Confirmation of Successful Reprogramming of OMLP-iPSCs by Differentiation Down Mesoderm, Endoderm, and Ectoderm Lineages

To test the reprogrammed oral iPSCs, their differentiation potential was investigated. EBs formed readily following 8 d in

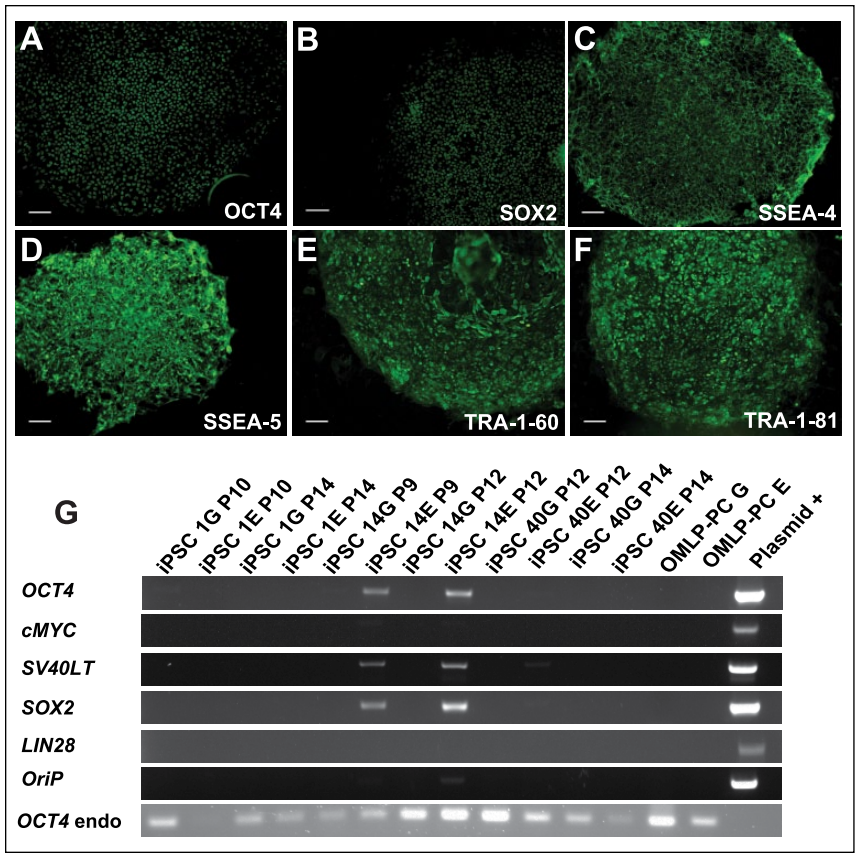

Figure 3. Characterization of oral mucosal lamina propria progenitor cells (OMLP-PCs). Presence of transcription factors (**A**) OCT4 in OMLP induced pluripotent stem cell (iPSC) colony. (**B**) SOX2. Presence of stem cell surface markers in OMLP-iPSC colonies (**C**) SSEA-4, (**D**) SSEA-5, (**E**) TRA-1-60, and (**F**) TRA-1-81. (**G**) Polymerase chain reaction analysis of presence of plasmid in E-episomal DNA and G-genomic DNA isolated from 3 OMLP-iPSCs (1, 14, 40) between passages 9 and 14. Episomal and genomic DNA isolated from OMLP-PCs were used as a negative control. Scale bars = 100 μm.

culture without bFGF on non-tissue culture plastic (Fig. 4A). To determine whether these EBs cultured from OMLP-iPSCs had the potential to form cells from all 3 germ layers, they were subjected to 3 distinct differentiation conditions. In all cases, EBs derived from each cell type demonstrated flattening out and outgrowth of cells from the original EB structure by day 14 (Fig. 4B). These cultures were stained for typical early stage markers of mesoderm, endoderm, and ectoderm. OMLP-PC-iPSCs were found to differentiate down early endoderm lineages by demonstrating positive production of α-1-FETOPROTEIN (Fig. 4C), early ectoderm lineages by the presence of βIII-TUBULIN (Fig. 4D), and the early mesoderm lineage by the positive presence of BRACHYURY (Fig. 4E). All IgG controls and secondary omission controls were found to be negative.

Discussion

Reprogramming with Plasmid Vectors

Despite OMLP-PCs expressing pluripotency markers prior to reprogramming, we established that, with the exception of *KLF4*, the expression levels of the markers were at far lower levels than in pluripotent hESCs. OMLP-PCs were, however, successfully reprogrammed through a non-integrating system consisting of 2 episomal plasmids expressing all 6 factors. The fact the *KLF4* expression was elevated compared with H9 suggests that the cells may be reprogrammed following removal of this factor, further enhancing their potential use in clinical applications. Colonies that possessed tight boundaries and consisted of cells with very high nuclear:cytoplasmic ratio—typical morphologic characteristics of hESCs (Thomson et al. 1998)—were identified and isolated. These colonies were positive for the transcription factors OCT4 and SOX2, which have been reported for both iPSCs and hESCs at the RNA and protein levels (Bhattacharya et al. 2005; Lowry et al. 2008; Maherali et al. 2008; Mali et al. 2008; Li et al. 2009). Additionally, the iPSC colonies were positive for the stem cell surface markers SSEA-4, SSEA-5, TRA-1-60, and TRA-1-81—markers not detected in OMLP-PCs, adding weight to the fact that these OMLP-iPSCs had potentially been reprogrammed. The presence of these typical stem cell markers—namely, SSEA-4, TRA-1-60, and TRA-1-81—in the iPSCs is consistent with the original research carried out for hESCs (Thomson et al. 1998) and results demonstrated for iPSCs by several research groups (Maherali et al. 2007; Takahashi et al. 2007; Miyoshi et al. 2010) confirming the presence of pluripotent stem cells. Moreover, the expression of SSEA-5 further confirms that these cells were in an undifferentiated state, given that expression of SSEA-5 is the first of the investigated markers known to be lost upon differentiation of the stem cells (Tang et al. 2011). When subjected to differentiation conditions, differentiated EBs positively expressed early markers of mesoderm (BRACHYURY), endoderm (α-1-FETOPROTEIN), and ectoderm (βIII-TUBULIN), consistent with previous reports for other iPSCs (Aasen et al. 2008; Maherali et al. 2008; Mali et al. 2008; Lin et al. 2009).

With respect to oral cell populations and reprogramming, fibroblasts from the oral mucosa have already been successfully reprogrammed; however, this was carried out with retroviral transfer of the genes (Miyoshi et al. 2010), which has been linked to formation of carcinomas and poses a clinical risk (Okita et al. 2007; Yamanaka 2007; Nakagawa et al. 2008). Our work suggests that reprogramming of oral PCs with non-integrating plasmids for transient expression of the

pluripotency factors is possible and practicable. To this end, we have confirmed that the plasmids did not integrate into the genome by identifying the absence of transgene sequences in the genomic DNA isolated from these iPSC lines via primers designed to incorporate a small sections of the plasmid (Fig. 3G). Furthermore, our results are consistent with those published following the use of these 2 plasmids to reprogram human dermal fibroblasts. These results demonstrated iPSC formation without integration of the plasmid, and only residual episomal presence of plasmids was identified (Yu et al. 2009). The presence of endogenous *OCT4* in both genomic and episomal DNA isolations is an indication of genomic DNA contamination of the episomal isolation; however, this does not significantly detract from the results demonstrated by absence of any plasmid in the genomic isolations. It is also interesting to speculate that an isolated PC population may represent a preferential source of cells for use in reprogramming, in line with the thoughts of others (Okita et al. 2007; Aasen et al. 2008). Indeed, studies have suggested that MSC-like cells isolated from dental pulp and exfoliated deciduous teeth are reprogrammed at a potentially higher efficiency (Yan et al. 2010). Hence, we postulate that the resulting iPSCs represent a potential source of cells that are therefore likely to be clinically safer for translational research. Indeed, in relation to this clinical translation, much work is currently underway to determine chemically defined conditions for the culture of isolated cells, for derivation, and for the onward culture of iPSCs to produce those that are ready for regenerative medicine applications (Chen et al. 2011; Takeda-Kawaguchi et al. 2014).

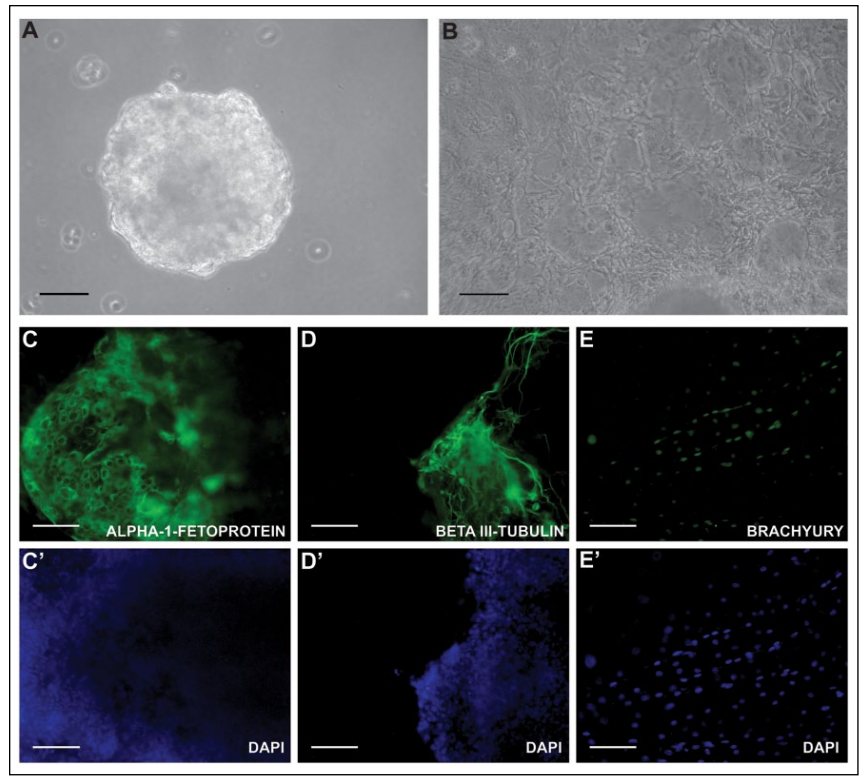

Figure 4. Differentiation capacity of oral mucosal lamina propria progenitor cells. (**A**) Embryoid body formation following 8 d in hydrophobic plates. (**B**) Phase image of embryoid body differentiation over 14 d. (**C**) Presence of α-1-FETOPROTEIN in differentiated (embryoid body) cultures. (**C′**) DAPI counterstain. (**D**) Presence of βIII-TUBULIN in differentiated EB cultures. (**D′**) DAPI counterstain. (**E**) Presence of BRACHYURY in differentiated EB cultures. (**E′**) DAPI counterstain. Scale bars = 100 µm.

Conclusion

The work shows that oral mucosa lamina propria PCs isolated from patients undergoing routine dental treatment can be reprogrammed to cells demonstrating typical stem cell markers and pluripotent characteristics of hESCs through a nonintegrating system. The resulting iPSCs demonstrated expression of early-stage markers of the mesoderm, endoderm, and ectoderm, confirming the potential for these cells to differentiate into cell types from all 3 germ layers. Future studies will address the clinical utility of such cell types for personalized regenerative medicine applications.

Author Contributions

R.A. Howard-Jones, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; O.K.Y. Cheung, contributed to data acquisition and analysis, critically revised manuscript; A. Glen, contributed to conception, design, and data acquisition, drafted and critically revised the manuscript; N.D. Allen, P. Stephens, contributed to conception, design, and data interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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