*Cell Prolif.* 2009, **42**, 587–594 doi: 10.1111/j.1365-2184.2009.00623.x

# Human immature dental pulp stem cells share key characteristic features with limbal stem cells

B. G. Monteiro*,†, R. C. Serafim‡, G. B. Melo§, M. C. P. Silva‡, N. F. Lizier*,‡, C. M. C. Maranduba*, R. L. Smith‡, A. Kerkis¶, H. Cerruti**, J. A. P. Gomes§ and I. Kerkis*

**Genetics Laboratory, Butantan Institute, São Paulo, SP, Brazil, †Department of Biotechnology, University of São Paulo, São Paulo, SP, Brazil, ‡Department of Morphology and Genetics, Federal University of São Paulo, São Paulo, SP, Brazil, §Department of Ophthalmology, College of Medicine, Federal University of São Paulo, São Paulo, SP, Brazil, ¶Applied Genetics, Veterinary Activities Ltda., Center of Innovation Technologies, São Paulo, SP, Brazil, **Clinic and Center of Odontology Research, São Paulo, SP, Brazil*

*Received* 23 *May* 2008*; revision accepted* 4 *October* 2008

## Abstract

*Objectives*: Limbal stem cells (LSC) are self-renewing, highly proliferative cells *in vitro*, which express a set of specific markers and *in vivo* have the capacity to reconstruct the entire corneal epithelium in cases of ocular surface injury. Currently, LSC transplantation is a commonly used procedure in patients with either uni- or bilateral total limbal stem cells deficiency (TLSCD). Although LSC transplantation holds great promise for patients, several problems need to be overcome. In order to find an alternative source of cells that can partially substitute LSC in cornea epithelium reconstruction, we aimed at investigating whether human immature dental pulp stem cells (hIDPSC) would present similar key characteristics as LSC and whether they could be used for corneal surface reconstruction in a rabbit TLSCD model.

*Materials*: We used hIDPSC, which co-express mesenchymal and embryonic stem cell markers and present the capacity to differentiate into derivative cells of the three germinal layers. TLSCD was induced by chemical burn in one eye of rabbits. After 30 days, the opaque tissue formed was removed by superficial keratectomy. Experimental group received undifferentiated hIDPSC, while control group only received amniotic membrane (AM). Both groups were sacrificed after 3 months.

*Results and conclusions*: We have demonstrated, using immunohistochemistry and reverse transcription–polymerase chain reaction, that hIDPSCs express markers in common with LSC, such as ABCG2, integrin β1, vimentin, p63, connexin 43 and cytokeratins 3/12. They were also capable of reconstructing the eye surface after induction of unilateral TLSCD in rabbits, as shown by morphological and immunohistochemical analysis using human-specific antibodies against limbal and corneal epithelium. Our data suggest that hIDPSCs share similar characteristics with LSC and might be used as a potential alternative source of cells for corneal reconstruction.

Correspondence: I. Kerkis, Av. Vital Brazil 1500, São Paulo–SP 05503-900, Brazil. Tel.: +55 11 3726 7222 R2287; Fax: +55 11 3726 1505; E-mail: ikerkis@butantan.gov.br

## Introduction

Limbal stem cells (LSC), which reside in the transition area between the cornea and the sclera, are capable of promoting constant renewal of the corneal epithelium and its regeneration in case of injury. When isolated, LSCs are observed to be self-renewing, highly proliferative cells *in vitro*, which express a set of specific markers, such as cytokeratins (K3/K12), gap junction proteins (connexins 43 and 50), integrins (β1 and α9), transporters (ATP-binding cassette, subfamily G, member 2, ABCG2), tumour-expressing protein p63, and others (reviewed in Pajoohesh-Ganji & Stepp (1) and Schlötzer-Schrehardt & Kruse (2)). LSCs have the capacity to reconstruct the entire corneal epithelium *in vivo* in case of ocular surface injury (1–13).

A variety of diseases, such as Stevens–Johnson syndrome, chemical burns and ocular cicatricial pemphigoid, may cause partial or total limbal stem cell deficiency (TLSCD). As a result of such deficiencies occurs the process of conjunctival epithelium invasion through the epithelium of the eye periphery, leading to a significant loss of visual acuity (13,14). Various strategies have been proposed for the treatment of TLSCD; these include transplantation of LSC autograft, oral mucosal epithelium, AM, autologous cultivated corneal ephitelium and other methods (13,15–25). Among them, autologous LSC

**Table 1.** Primers used for analysis of expression of LSC in hIDPSC

| Gene | Sense primer | Anti-sense primer | Annealing temperature |
|---|---|---|---|
| ABCG2 (379 bp) | AGTTCCATGGCACTGGCCATA | TCAGGTAGGCAATTGTGAGG | 55 °C |
| Connexin 43 (154 bp) | CCTTCTTGCTGATCCAGTGGTAC | ACCAAGGACACCACCAGCAT | 55 °C |
| K3 (145 bp) | GGCAGAGATCGAGGGTGTC | GTCATCCTTCGCCTGCTGTAG | 58 °C |
| K12 (150 bp) | ACATGAAGAAGAACCACGAGGATG | TCTGCTCAGCGATGGTTTCA | 58 °C |
| β-actin (208 bp) | GGCCACGGCTGCTTC | GTTGGCGTACAGGTCTTTGC | 55 °C |

transplantation from the healthy eye is a commonly used procedure; this, in turn, causes LSC deficiency in the healthy eye (7). In patients with bilateral LSC deficiency, heterologous LSC transplantation is used. However, in this case, rejection of the transplanted cells by the recipient environment may occur (26). LSC eye transplantation holds great promise for patients with TLSCD, although insufficient cell number for transplantation and rejection between donor cells and the recipient need to be overcome. Researchers are still working in order to find an alternative source of cells, which can partially substitute LSC in corneal epithelium reconstruction, especially in TLSCD patients.

Searching for an alternative stem cell source that could be potentially used in corneal reconstruction, we turned our attention to a population of stem cells recently isolated by our group from deciduous teeth, which were named human immature dental pulp stem cells (hIDPSC). These cells were shown to express both mesenchymal stem cell markers (SH2, SH3 and SH4) and human embryonic stem cell markers (OCT 4, NANOG, SSEA-3 and SSEA-4). They are also capable of undergoing differentiation into derivates of the three embryonic layers *in vitro* and of presenting notable engraftment into different tissues after their transplantation into adult mice, even without the use of any immunosuppression protocol (27,28). More recently, we have shown that hIDPSCs can survive and proliferate within the mouse developing blastocyst. They are capable of producing human/mouse chimaeras, as well as differentiating into human-specific tissues within the mouse embryonic environment (29).

In the present study, we aimed at investigating whether hIDPSCs could present similar key characteristics to LSCs and whether they could be used for corneal surface reconstruction, in a rabbit TLSCD model.

## Materials and methods

### *Cell culture*

Human IDPSC ($2n = 46$, XX) were isolated and previously characterized and cultured as described by Kerkis *et al.* (27). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 (1 : 1, Invitrogen, Carlsbad, CA, USA), supplemented with 15% foetal bovine serum (Hyclone, Logan, UT, USA), 100 units/ml penicillin (Gibco, Grand Island, NY, USA), 100 μg/ml streptomycin (Gibco), 2 mM L-glutamine (Gibco) and 2 mM non-essential amino acids (Gibco). Culture medium was changed daily and cells were replaced every 3 days. After reaching 80% confluence, cells were washed twice in sterile phosphate-buffered saline (PBS, Gibco; 0.01 M, pH 7.4), enzymatically traded with 0.25% trypsin/EDTA (Invitrogen) and seeded in 25-$cm^2$ plastic flasks (Corning, New York, NY, USA). All cultures were incubated at 37 °C in 5% $CO_2$ at a high-humidity environment. All experiments were performed using cells at the same passages (P6 and P7).

### *Total RNA extraction and reverse transcription–polymerase chain reaction analysis*

Samples of human limbal and corneal epithelium were donated by patients of the Department of Ophthalmology, Federal University of São Paulo, after informed consent and were used as controls in the present work. Total mRNA from these human limbal, corneal epithelium and hIDPSCs were extracted using TRIZOL reagent (Invitrogen) according to the manufacturer's protocol. Total mRNA was reverse-transcribed in cDNA using SuperScript First-Strand Synthesis System and Oligo $dT_{20}$ primer (both from Invitrogen). About 2 μl of mRNA was used per reaction. Reagents' final concentrations were 1× PCR buffer, 0.2 mM dNTP mix, 0.2 μM of each primer (Table 1); 1.5 mM of $MgCl_2$, and 2 units of Platinum *Taq* DNA polymerase (Invitrogen), with a final volume of 50 μl. PCR was performed using MiniCycler (MJ Research, San Francisco, CA, USA). Primary sequences and annealing conditions are shown in Table 1. PCRs were performed under the following conditions: 1 cycle at 94 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, at annealing temperature for 1 min, and at 72 °C for 1 min. PCR products were size-fractionated by 2% agarose gel electrophoresis and stained with 2% ethidium bromide (Sigma, St. Louis, MO, USA). Human β-actin gene was used as control.

### *Antibodies*

Mouse anti-human monoclonal antibodies: anti-integrin-beta 1 (integrin β1) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), connexin 43 (Chemicon), ABCG2 (Chemicon) and vimentin (NeoMarkers, Fremont, CA, USA), cytokeratin 18 (Sigma); and cytoplasmic/nuclear monoclonal antibodies: mouse anti-cytokeratin 3/12 (K3/12) (RDI, Flanders, NJ, USA), reacts with human and rabbit, and mouse anti-human anti-p63 (Chemicon). Mouse anti-human IDPSC antibody was obtained as described previously (27).

### *Immunofluorescence staining*

Cells were grown on glass cover-slips up to 70% confluence, washed in PBS (Gibco) and fixed overnight with 4% paraformaldehyde (Sigma). Coverslips were washed three times in tris buffered saline (TBS), containing 20 mM Tris-HCl pH 7.4 (Vetec, Duque de Caxias, RJ, Brazil), 0.15 M NaCl (Dinâmica Reagent, São Paulo, SP, Brazil), and 0.05% Tween-20 (Sigma). Permeabilization was performed using 0.1% Triton X-100 for 15 min (Santa Cruz Biotechnology). Cells were washed three times and incubated for 30 min in 5% bovine serum albumin (Sigma) in PBS pH 7.4 (Gibco). Primary antibodies were added for 1 h on each slide at different dilutions (connexin 43, ABCG2, vimentin, integrin β1 and K3/12 (1 : 100), p63 (1 : 200) and anti-hIDPSC (1 : 1000)), which were incubated at room temperature. Following washing in TBS (three times), cells were incubated in the dark for 1 h with secondary anti-mouse antibody-conjugated fluorescein isothiocyanate (FITC) at a dilution of 1 : 500. Microscope slides were mounted in antifade solution (Vectashield mounting medium, Vector Laboratories, Hercules, CA, USA) with 4′,6-diamidino-2-phenylindole (DAPI) and analysed using a confocal microscope. Control reactions were incubated with PBS instead of primary antibody, followed by washing and incubation with respective secondary antibody.

### *Confocal microscopy*

An argon ion laser was used, set at 488 nm for FITC and at 536 nm for Cy3 (Chemicon, Temecula, CA, USA) excitation. Emitted light was filtered using a 505-nm (FITC) and 617-nm (Cy3) long pass filter in a laser scan microscope (LSM 510 META, Carl Zeiss, Jena, Germany).

### *Animal TLSCD model*

All procedures were performed according to Institutional Ethics Committee requirements (Butantan Institute, protocol number 06/205). Male New Zealand white rabbits, weighing 2–2.5 kg, were anaesthetized through intramuscular injection of ketamine (10 mg/kg) and xylazine (2 mg/kg) and topical application of proxymetacaine. TLSCD was induced by chemically burning the right eye of each rabbit ($n = 5$). A filter paper saturated in NaOH 0.5 M was applied to the eye for 25 s, which was later irrigated with PBS. Dexamethasone-gentamicin solution was applied to each treated eye twice a day for 7 days, and pain was managed using midazolam (0.3 mg/kg, once a day for 1 week).

### *Preparation of hIDPSCs for transplantation*

Three days before surgery, hIDPSCs were seeded directly, without any feeder cell layer, at a density of $2 \times 10^6$ cells per temperature-responsive cell culture dish (35-mm diameter, Cell Seed Inc., Tokyo, Japan), which were kindly donated by Masayuki Yamato, PhD, of the Tokyo Women's Medical University (23,25). Cells were maintained in DMEM/F12 supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine and 2 mM non-essential amino acid, without foetal bovine serum, and were incubated at 37 °C in 5% $CO_2$ in a high-humidity environment until they rich confluence and formed a cell sheet. On the day of transplantation, viable cell sheets were harvested by reducing temperature of the culture to 20 °C for 30 min, following previously described protocol (13).

### *Transplantation of hIDPSCs*

After 1 month of injury, a 360° peritomy followed by a superficial keratectomy was performed under anaesthesia in order to remove fibrovascular pannus covering the animals' burned corneas. Subsequently, the harvested sheet of hIDPSC was placed directly onto the exposed transparent stromal bed as described previously (13,23,30). Afterwards it was covered with a patch of acellular human amniotic membrane (AM) and sutured using 10.0 nylon epithelial side down to the episclera, in order to avoid casual damage by the animal. In control animals ($n = 5$), denuded corneas were covered with AM only (17,19). In order to avoid pain, which could be provoked by surgery, the animals received subcutaneous injections of buprenorphine (0.05 mg/kg) once a day for 1 week. No other drugs were used, allowing evaluation of cell contribution without interference of other factors. It was not necessary to use any immunosuppressor as these cells are not rejected by the recipient (28,31). After 3 months, the animals were killed according to a protocol approved by the Butantan Institute, and their corneas were collected and processed for further analysis.

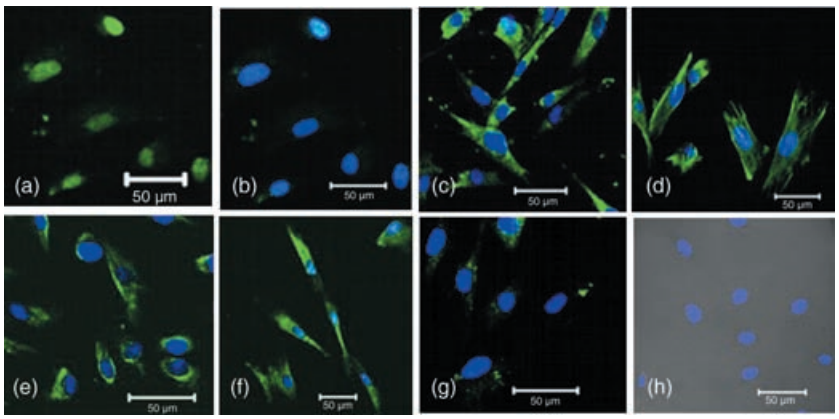


**Figure 1. Undifferentiated human immature dental pulp stem cells (hIDPSC) showing positive immunostaining with human-specific antibodies against limbal stem cells and corneal epithelium.** (a) Immunopositive reaction with p63 (green) in hIDPSC nuclear localization; (b) merged image, nuclei stained with DAPI; (c) integrin β1, presents membranic localization; (d) vimentin, intermediate filament; (e) connexin 43, cell membrane; (f) ABCG2, plasma membrane; (g) K3/12 antibody showed weak positive immunostaining; (h) negative control, hIDPSC, primary antibody was omitted, hIDPSC incubated with secondary antibody. Confocal microscopy: (a) Fluorescent microscopy (Fcm), (d–g) Fcm, (h) Fcm + differential interference contrast (Epi + DIC). Nuclei stained whit DAPI in blue. Scale bars = 50 µm.

*Histology*

For histological analysis, corneal tissues were fixed in 4% paraformaldehyde overnight and embedded in paraffin wax (Sigma), following the procedure of Du *et al*. (21). Corneal specimens were then cut (5 µm). Paraffin wax was removed from slides with xylene treatment and samples were rehydrated with ethylic alcohol in reducing dilutions. Histological images were captured using an Axio Imager A1 (Carl Zeiss, Jena, Germany) with an AxioCAM MRc5 (Zeiss) and processed with AxioVision software (Zeiss).

*Immunohistochemistry*

Corneal tissue samples were obtained from experimental animals that received transplantation of hIDPSCs and control injured animals, and were fixed with 4% paraformaldehyde and embedded with Tissue-Tek® O.C.T.™ (Sakura, Torrance, CA, USA). Frozen sections (5 µm) were obtained using a cryomicrotome (Cryostat CM1100, Leica, Wetzlar, Germany) and placed on poly-L-lysine-coated slides, which were incubated in cold methanol for 15 min in order to decrease tissue autofluorescence. Afterwards, they were washed three times in TBS and permeabilized with 0.1% Triton X-100 for 30 min. Slides were washed three times again and blocking solution 5% bovine serum albumin (Sigma) was added for 1 h. Primary antibody was diluted in PBS and slides were incubated for 1 h with integrin β1, cytokeratin 18 and K3/12 (1 : 50), p63 (1 : 100) and anti-hIDPSC (1 : 500) antibodies, at room temperature. Slides were again washed three times in TBS and incubated with secondary antibody Cy3 or FITC (1 : 500) in the dark for 1 h. After washing with PBS, slides were mounted in Vectashield (Invitrogen) with DAPI or propidium iodide (PI), and sections were examined under a confocal laser scanning microscope (LSM 510 META, Carl Zeiss, Jena, Germany) or a fluorescence microscope (Axio Imager A1, Carl Zeiss, Jena, Germany). Frozen sections obtained from rabbits, which received AM only after surgery, were used as control and were incubated with respective primary and secondary antibodies, following above-described protocol.

*Transmission electron microscopy*

Corneal tissue samples obtained from control normal and experimental animals that received transplantation of hIDPSCs were washed twice in PBS solution containing 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). Samples were washed three times in PBS and post-fixed for 15 min in 1% osmium tetroxide in PBS. Then they were dehydrated in ethanol and were embedded in Araldite resin. Ultrathin sections were cut using a Leica Ultracut ultramicrotome, stained with uranyl acetate and uranyl acetate, and observed under a transmission electron microscope (Philips, Morgagni 268).

## Results

*Expression of LSC and epithelial cell markers in undifferentiated hIDPSCs in vitro*

Using immunohistochemical analysis, presence of several LSC and epithelial proteins were evaluated in undifferentiated hIDPSCs. Human-specific antibodies, such as p63 (Fig. 1a,b), integrin β1, vimentin, connexin 43 and ABCG2, presented positive immunostaining with hIDPSC, while anti-K3/12 antibody showed only weak positive immunostaining in a few cells (Fig. 1c–h).

Expression of some genes previously analysed using immunofluorescent proteins was also accessed by reverse

 

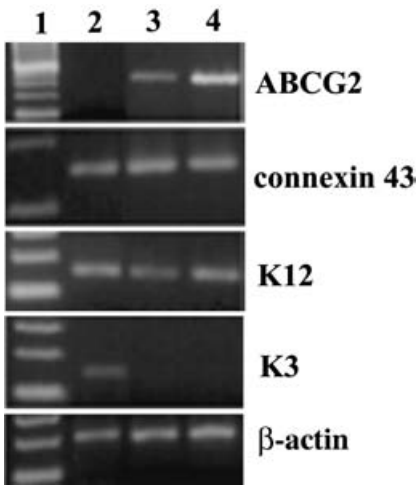


**Figure 2. Reverse transcription–polymerase chain reaction (RT-PCR) analysis of expression of limbal stem cell markers in human immature dental pulp stem cells (hIDPSC)**: Lane 1, ladder 100 bp; Lane 2, human corneal tissues; Lane 3, human limbal tissue; Lane, 4 hIDPSC. RT-PCR analyses demonstrated presence of ABCG2, connexin 43 and K12 mRNAs in hIDPSC, while those of K3 has been not found in hIDPSC.

transcription–polymerase chain reaction (RT-PCR). Undifferentiated hIDPSCs demonstrated expression of ABCG2, connexin 43 and K12, while expression of K3 was not found (Fig. 2, Lane 4). As expected, ABCG2, connexin 43 and K12 were expressed in human limbal tissue used as control, while mRNAs of K3 were undetectable (Fig. 2, Lane 3). In human corneal control samples connexin 43, K12 and K3 mRNAs, were present (Fig. 2, Lane 2). Predictably, expression of ABCG2 was not detected in human control cornea (Fig. 2, Lane 2).

### *Differentiation of hIDPSCs after their transplantation into TLSCD animals*

Experimental animals showed gradual improvement in corneal transparency, which was evaluated 1 and 12 weeks after hIDPSC transplantation, respectively (Fig. 3a,b). Control animals, that had received AM only, 3 months later did not show any signs of amelioration and presented total conjunctivization of the eye surface and strong vascularization

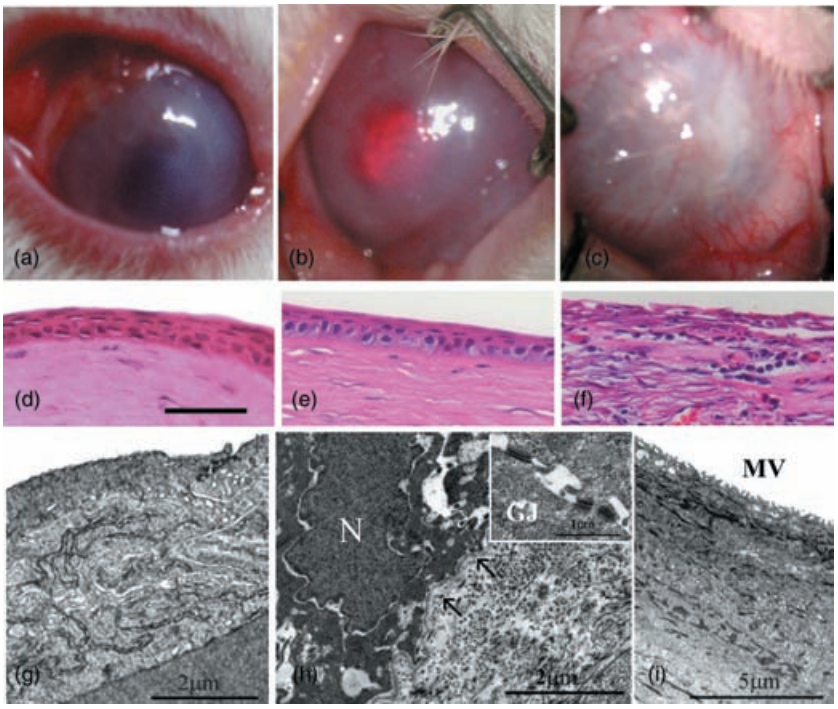


**Figure 3. Representative figure of reconstruction of rabbit's corneas with human immature dental pulp stem cells (hIDPSC) after alkaline burning.** (a,b) Ocular surface reconstruction in rabbits observed 1 and 12 weeks after the transplantation of hIDPSC, respectively. In (b), amelioration of corneal epithelium can be observed. (c) Ocular surface of the animals of control group, which received AM only, 12 weeks after surgery. Eye showed vascularization and was totally covered by conjunctiva. Histological analysis: (d) Control corneas obtained from non-injured eyes of the animals. (e) Corneal epithelium obtained from experimental animal after eye surface burning and tissue engineered hIDPSC sheet transplantation, showing morphologically well-formed structures of stromal layer and stratified epithelium. (f) Same as in (e) treated only with AM. Presence of vascular endothelium in stroma and a disorganized epithelium were observed. Light microscopy. Scale bars = 100 μm. Transmission electron microscopy of rabbit cornea (g) and cornea reconstructed with hIDPSC (h,i). Corneas reconstructed with hIDPSC showed presence of cell-basement membrane hemidesmosomes (arrows) and desmosomes in (h) (inset; GJ, gap junction), as well as microvillae (MV) on their surface. N, nucleus. Scale bars: (g,h) = 2 μm; inset in (h) = 1 μm; (i) = 5 μm.

 

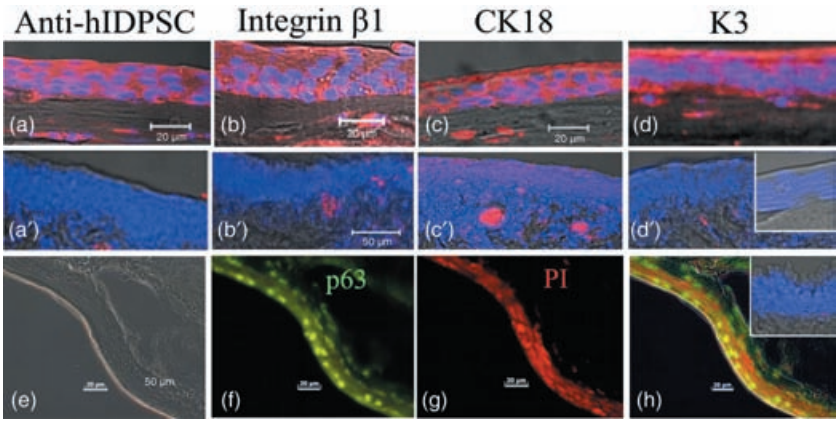


**Figure 4. Immunohistochemical staining in reconstructed corneas with human-specific antibodies.** (a–d) Experimental group: (a) Presence of human immature dental pulp stem cells (hIDPSC) in the newly formed cornea in the animal model was confirmed by positive anti-hIDPSC antibody immunostaining (red); differentiation of hIDPSC in cornea is evidenced by positive immunoreactivity with human-specific antibodies (red): integrin β1 (b), cytokeratin 18 (c), and K3/12 (d). (a′–d′) Control group, which received AM only, did not present positive immunostaining with these antibodies. Inset in (d′) shows negative control (primary antibody was omitted) hIDPSC incubated with secondary antibody. Nuclei stained whit DAPI (blue). (e–h) Experimental group: (e) Phase contrast image of cornea, (f) positive immunostaining with p63 antibody (green), (g) nuclei stained with PI (red). (h) Merged image of (f) and (g) (yellow). Inset in (h) presents lack of p63 antibody expression in cornea of control-group animal. (a,a′–d,d′) Confocal microscopy: merged image fluorescent microscopy + differential interference contrast. (e) Phase contrast; (e–g) fluorescent microscopy; (h) merged image fluorescent microscopy + phase contrast. Scale bars: (a–d, e–h) = 20 μm; (a′–d′) = 50 μm.

(Fig. 3c). Histological analysis of both corneas obtained from non-injured eyes of the animals and those that received hIDPSC sheet transplantation showed morphologically well-formed structures of stromal layer and stratified epithelium (Fig. 3d,e). In contrast, in control animals, which received AM only, corneas were chaotically organized and did not present any evidence of epithelialization (Fig. 3f). Transmission electron microscopy showed basal cells of corneal epithelium present with low nucleus to cytoplasm ratio. Corneal surfaces rely on presence of microvilli and cell–cell junctions, such as desmosomes, were also observed (Fig. 3g–i).

### *Evidence of hIDPSC differentiation and their contribution to ocular surface reconstruction in TLSCD animals*

In order to evaluate whether corneas formed in the injured rabbits' eyes originated from hIDPSCs, immunohistochemical analysis was performed using anti-hIDPSC antibody, which was obtained and previously characterized (27,29). We observed that this antibody reacted positively with the corneal epithelium, that had received hIDPSC transplantation, suggesting their human origin (Fig. 4a). In addition, expression of several anti-human antibodies, such as integrin β1, cytokeratin 18 and p63, which have nuclear localization, confirmed this result (Fig. 4b,c,e–h). Differentiation of hIDPSCs into corneal tissue *in vivo* was also evidenced by positive immunostaining with the antibody against corneal epithelium K3/12 (Fig. 4d,d′). As anticipated, none of the antigens/antibodies were detected in control animals, which received AM only (Fig. 4a′–d′).

## Discussion

Stem cells isolated from dental pulp are a promising source of cells to be used in cell therapy (27,31–33). Previously, we have shown that hIDPSCs are self-renewing, rapidly proliferating cells *in vitro*, which express different markers of both mesenchymal and embryonic stem cells. They are capable of undergoing differentiation into smooth and skeletal muscles, neurons, cartilage and bone *in vitro*; upon differentiation, these cells loose expression of embryonic stem cell markers. They also showed engraftment into various tissues after transplantation into mice, do not form tumour and are not rejected by recipient organisms. These cells do not have the necessity for any feeder layers, facilitating possible transition of this protocol to clinical use (27). More recently, we have demonstrated that hIDPSCs were capable of reconstructing large cranial defects in non-immunosuppressed rats, and clinical amelioration of muscular dystrophy in golden retriever dogs was observed after eight consecutive early transplantation of hIDPSCs, with no prior immunosuppression (28,31). We have demonstrated here that they also express several LSC markers when cultured *in vitro*, and can reconstruct the ocular surface in the case of injury in animal models, producing well-formed corneal epithelium.

 

LSC populations are highly heterogeneous and are composed of suprabasal and basal limbal cells, which differ in their differentiation potentials (2). It is believed that populations of suprabasal limbal cells have a limited number of cell divisions before they become terminally differentiated (1,2,5). Expression of such markers as vimentin, integrin β1, and p63 have been reported for both basal and suprabasal limbal cells. ABCG2 was expressed only in basal limbal cells, while K3, K12 and connexin 43 were frequently observed in suprabasal limbal cells (2,11,34,35). We selected the aforementioned markers, as they were more frequently used to characterize LSC populations. We showed using immunofluorescence and RT-PCR analysis, that hIDPSCs expressed such selected LSC markers as ABCG2, connexin 43, integrin β1, p63 and vimentin. Gene expression profiles of putative human LSC cultivated *in vitro* have been described previously and are in accordance with results obtained in the present work (34). In addition, it has also been reported that rat dental pulp cells cultivated *in vitro* showed expression of connexin 43 (36) and that expression of ABCG2 was found inside population of dental pulp stem cells (37).

The expression of K3/12 is thought to be a hallmark of epithelial cells with corneal-type differentiation (38). In the present study, two separate primers for genes K3 and K12 were used for RT-PCR analysis. As expected, the K12 primer was amplified by RT-PCR in undifferentiated hIDPSCs, while K3, which is a marker of terminally differentiated corneal epithelium, did not present such amplification (38). We observed weak positive immunostaining with K3/12 antibody in undifferentiated hIDPSCs, which is mainly due to their positive response to the K12 epitope of K3/12 antibody.

We tested the capacity of hIDPSCs to differentiate into corneal epithelium *in vivo*. Our results showed that these cells were capable of restoring corneal clearness and smoothness, and the entire corneal surface were completely composed of transplanted hIDPSCs. This is similar to results observed in various studies using an LSC population, oral mucosal epithelial stem cells, and mesenchymal stem cells (7,8,13). The process of corneal transparency began from the second week after transplantation and showed gradual amelioration over 3 months. At that moment, histological studies (haematoxylin & eosin) showed well-formed corneal epithelium similar to normal, while those from the control group (received AM only) presented a disorganized epithelium with inflammatory cells and vascular endothelium. Human mesenchymal stem cells isolated from bone marrow has also been tested as a candidate for treatment of ocular deficiency in rats. In contrast to our study, although these cells were capable of forming corneal epithelium, Ma and co-authors did not observed any clarity or expression of corneal markers (24). However, they did not investigate expression of LSC markers in undifferentiated human mesenchymal stem cells prior to transplantation, which makes it difficult to compare the results obtained in these two pieces of work.

In accordance with the definition of LSC, presented in the Introduction (1,2,7–9,39), one can conclude that hIDPSCs, used in the present work demonstrated similarities with LSC in gene expression profile as well as in the capacity to differentiate into corneal epithelium. Our results also support the hypothesis that stem cells, in particular hIDPSCs, can express markers of other undifferentiated stem cells (40,41). Our findings qualify hIDPSCs as an alternative source of cells from LSC, which can be potentially used for corneal reconstruction.

## Acknowledgements

The authors thank Dr Masayuki Yamato of Tokyo Women's Medical University for donating the scaffolds. We also thank Drs Toshie Kawano and Alexsander Seixas de Souza from the Laboratory of Parasitology of Butantan Institute for permission and technical instructions with confocal microscope. Additionally, we also thank Zenaide Lúcia da Silva, Vera Lúcia Aparecida Rodrigues de Farias and Antônio Fagundes for their technical assistance. This work was financially supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Clinic and Center of Odontology Research.

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