EXPERT REVIEWS

Skin and corneal cellular therapy using embryonic stem cells: how far are we?

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Adult stem cell therapy is efficiently applied to severe skin burn patients but with some important limitations. A major challenge remains with regards to the maintenance of the stem cell's self-renewal and pluripotency *ex vivo*. Human embryonic stem (ES) cells that are derived from the inner cell mass of the developing blastocyst were successfully isolated for the first time in 1998. These cells can be passaged in culture as undifferentiated pluripotent colonies and may also enter lineage-specific differentiation under the appropriate signals. Thus, human ES cells are considered to be a promising source for future regenerative medicine. Herein, we review the most recent advances and achievements as well as important challenges and obstacles that must be resolved before using ES cells for regenerative medicine of cutaneous and corneal epithelium. Recently, ES cells have been successfully differentiated into pure progenitor cell populations of epidermal lineage but future attempts are still required for manipulating these cells into their correct functionality *in vivo*.

KEYWORDS: cell therapy • cornea • corneal commitment • embryonic stem cells • epidermal commitment • keratinocytes • limbus • p63• Pax-6 • pluripotency • skin

Skin & corneal structures & characteristics

The skin and the cornea are both located at the outermost cell layer of the body and act as a protective barrier against infections and injuries [1–3]. These tissues appear to possess critical physiological and functional differences but, at the same time, share many similarities in terms of structures, functions and developmental origin [4–7]. The stratified keratinized skin epithelium (epidermis) and the stratified nonkeratinized corneal epithelium are both developed from the surface ectoderm, and both rest on a basement membrane that separates them from an underlying mesenchymal dermis or the corneal–stroma, respectively. The latter is bottomed by a monolayer of endothelium, which plays an essential role in the maintenance of corneal transparency [8], and the skin appendages (e.g., hair follicles, sebaceous glands and sweat glands), which are formed and distributed in the epidermis, are absent in the cornea.

The skin dermis and the corneal stroma are more than simple supportive tissues for their contiguous epithelia. Their functional instructive properties have a critical influence on their nearby epithelia. *In vivo* studies have clearly demonstrated that the type of epithelial differentiation is specifically dictated by the particular origin of its underlying dermis [9]. In this sense, it is worth noting that the skin dermis is vascularized, while the corneal stroma is not. Remarkable evidence that strengthens the notion of resemblances between the skin and the cornea is the fact that the corneal epithelium may become skin-like in a number of corneal pathologies [2,10–12]. Several corneal dystrophies are linked with corneal neovascularization, which, in some cases, is accompanied by corneal to epidermal-like transformation, loss of corneal transparency and, finally, partial or complete loss of vision [2,13]. Interestingly, corneal epithelium may transdifferentiate into epidermis under the influence of embryonic dermis [10–12], in the absence of Notch1 [14] or by the loss of DKK2 expression [15].

Epidermal & limbal stem cells

Both skin and cornea are extraordinary, dynamic, renewing tissues that rely on similar, if not nearly identical, keratinocytes stem cells for tissue replenishment [3,4,6,16–20]. The epidermal and corneal tissue turnover is 3–4 weeks and

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approximately 10 days, respectively. Various skin stem cell populations were localized to the bulge region of the hair follicle, the basal layer of the interfollicular epidermis and other skin compartments (reviewed in [4,7,16,21]). However, it has been believed for decades that corneal stem cells are restricted to the 'limbus' – a narrow ring-shaped zone located at the conjunctival transitional region, adjacent to the boundary between the cornea and the conjunctiva [2,3,18,22,23]. Both epidermal and limbal stem cells express the cytokeratin pair of K5 and K14. In the epidermis, the epidermal stem cells give rise to transit amplifying (TA) cells that proliferate, migrate and terminally differentiate, along with the substitution of K5/K14 with K1 and K10. The corneal (limbal) stem cells give rise to TA cells, which are thought to migrate from the corneal periphery while they proliferate and terminally differentiate and replace the cytokeratins K5/K14 with K3 and K12. Interestingly, it was recently shown that mice were able to repair corneal epithelial injury even after limbal stem cells were completely ablated using an ophthalmic cautery [14]. This observation implies that, at least in the mouse, another additional corneal stem or progenitor cell population exists, in a nonlimbal region.

Challenge of epidermal & corneal stem cell purification & expansion *ex vivo*

Many attempts have been made to find novel strategies for treating various skin and corneal disorders. The most exciting is the application of adult stem cells for autologous cellular therapy. This requires the extraction of stem cell-enriched epidermal cells from a patient, followed by their expansion *ex vivo*, genetic manipulation, if necessary, and finally retransplantation. Numerous studies have attempted to identify a single marker or a combination of molecular markers that are specifically expressed by epidermal or limbal stem cells. Several candidates were proposed as epidermal stem cell specific (e.g., integrin-β1 [24], K15 [25,26], K19 [27], high integrin-α6 coupled with low CD71 [28,29], absence of CD34 [30]) or as limbal stem cell specific (e.g., p63 [31], integrin-α9 [32], ABCG2 [32,33], C/EBPδ [34], and absence of either K3, K12 or connexin 43 [32]). The classical methods that are used to identify stem cells are based on their slow cell cycling *in vivo* (namely 'label-retaining cells' [LRCs]; described in detail in [16,35–38]) or on their high proliferative potential *in vitro* (namely 'holoclones' – generating cells by clonogenicity assay, reviewed in [39,40]) or on their ability of excluding the nuclei dye of Hoechst 33342 when analyzed by flow cytometry (namely 'side population [SP] cells'; detailed in [33,41–44]). These methods enable the identification and localization of epidermal and corneal stem cells in different cutaneous niches [27,37,45], as well as in the limbus [33,46,47]. However, neither LRC, nor SP cells, which are both nuclei-labeled cells, are compatible for clinical purposes. As for holoclones, it is not yet known how to grow them *in vitro* without reducing their pluripotency. Interestingly, it is possible that stem cells isolation by each one of the above-described techniques would give rise to the enrichment of nonredundant stem cell populations [48].

Since allogenic keratinocytes are rejected by the host, skin allografts can essentially serve as coverage for the short term [49]. On the other hand, transplantation of reconstituted skin that is carried out by autologous epidermal cells has already been used for decades for treating extensive deep skin burns, chronic wounds and ulcers [50,51]. Recently, De Luca *et al.* have reported the treatment of a patient affected by junctional epidermal bullosa with inverted autologous epidermis [52]. Patient-derived cultured epidermal stem cells were transduced with a retroviral vector encoding for the wild-type laminin-5 β3 gene and the resulting corrected epidermal sheets were transplanted back onto the patient's legs. Over 1 year after grafting, the retroviral containing skin still persisted, strongly suggesting that stem cells were successfully transduced. Autologous reconstituted skin grafting approach is successful and has saved patients worldwide. However, skin appendages (e.g., hair follicles, sweat glands and sebaceous glands) are absent in the reconstituted skin, although their presence is essential for long-term tissue replenishment and complete skin physiological functions, as well as for graft aesthetic. Thus, an important challenge remains with regard to the nature of the signals; perhaps embryonic signals are required to maintain the complete pluripotency of stem cells in our culture dish. In addition, the stage of skin stem cell amplification *ex vivo* is time-consuming as it requires their extraction from a patient, followed by their expansion *ex vivo* and therefore is often unsuitable for patients that need urgent treatment. For these patients, the need for an immediate cellular source of cells that are 'ready to use' remains crucial.

Corneal allotransplantations are very common but the shortage of donors and the high prevalence of corneal rejections, especially in the long term, have encouraged the investigation of new treatments [13,53–56]. Limbal autograft transplantation can successfully restore vision of patients suffering from ocular surface diseases [57]. However, this technique requires relatively large limbal biopsies from the healthy eye and is not applicable for patients with bilateral lesions. Successful corneal repair was obtained by *ex vivo* expansion of limbal epithelial cells [58,59]. In this method, autologous limbal epithelium is harvested from contralateral healthy eye or from a normal limbal region of the eye in case of a partial stem cell deficiency, and is then expanded *ex vivo* to obtain transplantable culture sheets. This powerful technique has been successfully applied by several groups worldwide [60–62]. For patients with total limbal stem cell deficiency in both eyes, autologous limbal stem cell expansion is not possible and therefore allogenic limbal stem cell expansion can be applied. Interestingly, the successful *ex vivo* expansion and corneal transplantation of autologous oral mucosal epithelial cells has been reported [63–65]. However, in some corneal dystrophy, such as Stevens–Johnson syndrome, these approaches are sometimes incompatible since the disease

may involve other epithelia, including oral mucosal epithelium [13,53]. In these cases again, it is necessary to develop novel therapies and the search for alternative cellular sources, such as embryonic stem (ES) cells, becomes critical.

Therefore, the generation of ES-derived progenitor cells as an alternative cellular source may be invaluable for both skin and corneal therapy. Moreover, the elucidation of the molecular mechanisms that underlie stem cell self-renewal may facilitate the maintenance of the stemness phenotype of adult stem cells in culture.

ES cells for skin & corneal cellular therapy

Embryonic stem cells are maintained in culture as undifferentiated pluripotent colonies but, under specific conditions that recapitulate developmental signals, may give rise to different progenies representative of the three embryonic layers: the endoderm, the mesoderm and the ectoderm [66]. In the last decade, many attempts have been made to optimize the culture conditions for differentiating ES cells toward a wide range of specific tissue lineages, including skin and corneal lineages. These progressions are clearly significant steps forward. However, in most cases, those models may only serve as excellent *in vitro* cellular models for studying embryonic development and postnatal maintenance of the skin and cornea. As described below, several issues must be resolved before ES cell therapy becomes a reality.

Derivation of epidermal precursors & skin formation from mouse ES cells

The first demonstration that ES cells can produce keratinocytes was performed by Watt *et al.* [67,68]. Murine ES cells were permitted to grow as embryoid bodies (EBs) for 9 days and then cultured on dead de-epidermized human dermis (DED) for 16–26 days. In these conditions, cysts of stratified epithelia that contained keratinocytes were detected by immunostaining [67]. The keratinocyte differentiation was not uniform but patched among various other types of differentiating cells, as expected from EB cultures. Nevertheless, this system allowed tailoring of the involvement of β1-integrin in keratinocyte differentiation. It was further demonstrated that β1-integrin-deficient ES cells failed to differentiate into keratinocytes in contrast to wild-type ES cells. This phenotype was partially restored by the cultivation of living fibroblasts on the DED, or even at the presence of fibroblast-conditioned medium. A complete recovery in the ability of β1-integrin-deficient ES cells to induce keratinocyte differentiation was achieved when specific fibroblast-secreted growth factors, such as KGF and FGF-10, were present.

Turksen and Troy have also demonstrated the derivation of mouse ES cells to epidermal cells through a two-step process [69]. Epidermal progenitors were first derived from EBs and were further co-cultured on basement membrane matrix (growth factor-reduced Matrigel™) [69] or, more recently, on adult epidermal cells [70]. The resulting murine ES-derived epidermal progenitor cells were significantly enriched. However, late-stage and terminal-stage of epidermal differentiation occurred at minor frequency and the capability of these cells to form stratification has not been further examined.

To evaluate the functionality of murine ES-derived keratinocytes, our group designed an alternative protocol of differentiation [71]. ES cells were cultivated on an acellular extracellular matrix in the presence of the bone morphogenetic factor (BMP)-4 and then subjected to air–liquid interphase for organotypic reconstitution. Remarkably, the resulting ES-derived organotypic tissue comprised a pluristratified squamous epithelium coupled with an underlying dermis. As demonstrated by immunofluorescence and electron microscopic analysis, the resulting bioengineered skin structure essentially resembled natural mouse embryonic skin, containing both epidermal and dermal compartments. Characteristics of each skin layer were present, including K14 and K10 located, as expected, at their respective epidermal layer. Several additional markers (collagen IV, collagen VII, laminin-1, laminin-5 and integrin-α6β4) were correctly located at the basement membrane zone, with distinct hemidesmosome anchorage structures, visualized by electron microscopy. The presence of nidogen, which is specifically produced by dermal fibroblasts, confirmed the dermal nature of the underlined compartment [71]. This study demonstrated for the first time that a complete bioengineered skin comprised of a well-ordered dermis and epidermis could be produced from ES cells *in vitro*. However, since the differentiated cell population was not homogenous (as it contains both keratinocytes and fibroblasts but also other cells committed to alternative lineages), the reconstituted skin was infrequently produced and could not be used for *in vivo* grafting assays. Alternative culture conditions or cell sorting in the case of novel specific cell surface markers, should be designed in order to obtain pure ES-derived epidermal cells that could be evaluated in transplantation (see below for human ES). Nevertheless, this cellular model provides an exceptional tool for investigating the embryonic development of both dermis and epidermis, and challenges the function of particular genes in these critical events. Accordingly, we recently delineated some molecular mechanisms of BMP-4 [72] and p63 [73] functions during ES neuro-ectodermal fates.

Attempts to purify & amplify keratinocytes from human ES cells

These models of producing keratinocytes from murine ES cells are certainly suitable and fruitful for fundamental study purposes. However, with regard to cell therapy, the demand for a homogeneous population that is derived from human ES cells is unavoidable. Therefore, the challenges of obtaining human ES-derived keratinocytes for epidermal reconstitution continue to attract much attention. Several studies that are described below are also summarized in Figure 1.

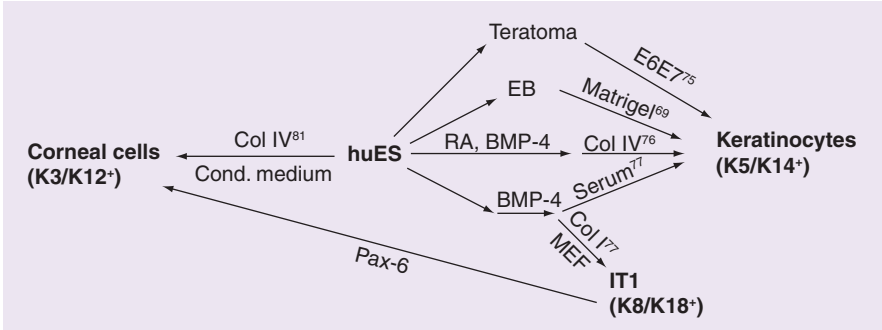

Figure 1. Different protocols describing epidermal and corneal differentiation of human embryonic stem cells. The studies are referred to in the text.
BMP: Bone morphogenetic protein; EB: Embryoid body; ES: Embryonic stem cell.

Green *et al.* focused on the challenge of producing human ES-derived keratinocytes through EBs or ES-derived teratoma [74,75]. In both cases, the epidermal populations were still highly heterogeneous, but could be somehow recovered and further cloned. However, the isolated keratinocytes stopped growing and quickly entered senescence. When differentiated EBs were further subcultured on 3T3 feeder cells in the presence of EpiLife® medium, most nonkeratinocytes were eliminated [75]. Since keratinocyte proliferation was not significantly changed, the cells could not be expanded in culture to any further extent. Finally, since other sophisticated alternatives did not appear to encourage growth of keratinocytes, the facilitation of keratinocyte expansion was carried out by immortalization by the transduction of the *E6E7* gene. Clonally derived lines of immortalized keratinocytes sustained keratinocyte-specific markers but the frequency in which they differentiated was lower than that of adult keratinocytes [75]. While this study described novel culture methods for studying and purifying keratinocytes from ES cells, immortalization is obviously not acceptable in terms of cell therapy approach. It does not solve the previous major concerns of how epidermal stem cells could be grown *in vitro* without losing their self-renewal and pluripotency.

Recently, Palecek *et al.* reported that a relatively pure population of keratinocytes could be derived when human ES cells are cultivated in the presence of retinoic acid and BMP-4, followed by additional subculturing on collagen IV [76]. Although this differentiation process is particularly long, it was shown to be highly efficient, resulting in an almost pure keratinocyte population. However, again, these ES-derived keratinocytes rapidly reached senescence within approximately ten population doublings and, unfortunately, the authors did not challenge the resulting pure population to an organotypic reconstitution assay.

Hence, the different groups that have succeeded in producing homogeneous ES-derived keratinocytes have also reported difficulties in growing them [75,76]. To overcome this problem, we have recently designed a new protocol for the isolation and amplification of ectodermal cells, which are the embryonic progenitors of a large number of somatic epithelial cells, including epidermal cells [77]. The generation of such ES-derived early progenitors could possibly serve as an efficient starting point for their differentiation into various ectodermal-derived tissues. Human ES cells were grown on fixed PA6 human fibroblasts. In the absence of serum, this protocol led to efficient production of dopaminergic neural cells [78]. BMP-4 is a morphogen demonstrated to play a critical role *in vivo* for epidermal differentiation of neuro-ectodermal progenitors [79,80]. In our protocol, human ES cells were grown on PA6 fibroblasts in the absence of serum and treated by BMP-4 from day 4 for 3 days. At day 7, serum addition resulted in a heterogeneous population comprised of K8/K18$^+$ ectodermal progenitor cells (60%) and K5/K14$^+$ epidermal-like cells (20%). Remarkably, by seeding this heterogeneous cell population on collagen I-coated dishes, a pure population of human ectodermal progenitors was obtained, all positive for K8 and K18 (referred to as IT1 cells) [77]. Cells grew relatively quickly, while ectodermal markers remained stable. Interestingly, we demonstrated that these cells, although derived from ES cells, behaved like somatic cells. They no longer expressed the pluripotency-specific transcription factor Oct-4, they displayed a somatic cell cycle profile with a normal G_1 phase and accordingly did not produce teratoma. Finally, it was possible to efficiently grow and amplify them before they reached senescence after approximately 60 population doublings. Moreover, these ectodermal precursors appear to be multipotent as they are able to differentiate *in vitro* into both K5/14$^+$ keratinocytes [77] and K3/12$^+$ corneal cells following exogenous expression of p63 and Pax-6, respectively (Shalom-Feuerstein R, Aberdam D, Unpublished Data). This differentiation protocol will therefore be useful as an *in vitro* cellular model that recapitulates the early steps of epithelial–embryonic development. Together, these findings demonstrate for the first time the highly reproducible isolation of a stable, homogeneous ectodermal cell population from ES cells through simple and defined culture conditions. In contrast to the clear difficulties in expanding keratinocytes *in vitro* [75,76], these cells can be passaged in culture to a relatively high extension before entering senescence [77]. Moreover, these cells overcome two of the main limitations related to human ES cells for cell therapy: unlimited cell growth and tumorigenicity. Hence, these cells may potentially serve as an unlimited source of safe and homogeneous multipotent ectodermal precursors in clinical trials.

ES-derived corneal cells

As mentioned previously, patients that entirely lost their limbal stem cells in both eyes due to inherited disease or chemical burns have less option of autotransplantation. In addition,

many recipients of corneal allografts undergo ocular inflammation, corneal neovascularization and irreversible rejection despite topical immunosuppression. However, regardless of the large necessity for alternative cellular sources for corneal transplantation, only three studies have reported the derivation of corneal–epithelial cells from ES cells [81–83]. In all three cases, a heterogeneous ES-derived $K12^+$ corneal cell population was obtained. In the first study, the derivation of corneal-like epithelial cells was obtained by culturing murine ES cells as EBs for 8 days on collagen IV-coated plates [82]. The corneal differentiation was documented by monitoring the corneal-specific cytokeratin K12. It was shown by RT-PCR and western immunoblotting that K12 mRNA levels and perhaps some protein levels were enhanced during ES cell differentiation. Owing to the inability of quantitative analysis (e.g., fluorescence-activated cell sorting analysis or at least immunofluorescent staining) for cytoplasmic K12, it was not possible to evaluate the prevalence of the corneal cells among the total differentiating cells [82]. Nonetheless, the ES-derived cells were examined *in vivo*. Mouse corneal surface injury with *N*-heptanol was followed by transplantation of the murine ES-derived cells into the injured cornea. Within 24 h of transplantation, complete re-epithelialization of the corneal surface occurred. However, any longer chase after transplantation was not demonstrated: neither a convincing demonstration that the cells were not derived from the wounded host, nor any examination of visual rescue.

In another recent study, human ES cells were directed to corneal fate by precise culture conditions that were designed to mimic the limbus microenvironment [81]. Similar to the previous study, human ES cells were seeded on collagen IV-coated dishes, but this time conditioned media of human limbal fibroblasts was added. Within only 6 days, a significantly large fraction of $K3/12^+$ corneal cells was detected. However, this corneal marker drastically and continuously declined with further differentiation, perhaps indicating that these cells were lost. Interestingly, along with corneal differentiation, epidermal differentiation occurred in parallel. In fact, p63 expression, which peaked at day 6, was followed by the appearance of terminally differentiated skin-like epithelial cells that expressed K10 at day 18–21 of differentiation [81]. No further data were presented regarding the apparent ectodermal and epidermal differentiation (i.e., K8/18 and K5/14). Nevertheless, this study clearly brought significant refinements to the culturing conditions that induce corneal cell differentiation from human ES cells, quickly and efficiently. It is an absolutely useful *in vitro* cellular model. Although, for unknown reasons, the prevalence of the corneal cells is declining, it may be interesting to evaluate the ability of these cells, perhaps in an earlier culture stage, to reconstruct a corneal epithelium *in vivo* or at least by organotypic culture. In this study, however, there was no documentation of the expression of Pax-6 [81], a critical transcription factor that is involved in eye development [84–86]. Pax-6 is known to specifically enhance K12 promoter activity [87] and its downregulation is linked to corneal disorders [88]. As described earlier, we recently reported a reproducible protocol for the isolation of a pure ectodermal population from human ES cells [77] that are able to efficiently differentiate further into corneal cells by exogenous expression of Pax-6 (Shalom-Feuerstein R, Aberdam D, Unpublished Data). Interestingly, seeding these cells on collagen IV-coated plates was sufficient to induce an elevation in Pax-6 expression (Shalom-Feuerstein R, Aberdam D, Unpublished Data). Grafting them onto animal eyes is the next challenge.

Expert commentary

Human ES cells are considered to be a promising source for regenerative medicine. Dozens of independent cell lines are now available worldwide and some have already been derived free of feeders or any animal products. The massive evolution in the field of ES cell research and several remarkable achievements give hope that, at least for several neurodegenarative diseases and cardiac defects, committed ES cells will be soon tested in human clinical trials. Concerning skin therapy, the manipulation of ES cells into a progenitor cell population of epidermal lineage has been reported. Both pure ES-derived keratinocyte [76] and ectodermal [77] cells, and also corneal [81] cells, which were discussed earlier (Figure 1), are clearly striking recent improvements of the previous techniques. However, it is not yet evident whether those cells can retain their stemness in the described cultures and, more importantly, whether they are able to produce a fully developed tissue. Further studies must thus be carried out to carefully address the potential of these ES-derived epidermal and ectodermal cells to form stratified epithelia *in vitro* and then be transplanted into animals. The obvious advantage of such an unlimited cellular source would be to provide a ready-to-use tissue source that can be held on standby for cell therapy application. It will be essential to compare the ES-derived epidermal and corneal cells with their somatic counterparts by broad-spectrum molecular approaches (i.e., transcriptome, proteome and miRnome). However, it is well known that ES cells possess, to some extent, oncogenic-like properties. The injection of undifferentiated ES cells into immunodeficient mice results in the formation of teratoma [89,90]. In order to make the use of ES cells for cellular therapy possible, the risk of teratoma formation must first be excluded. Additionally, the immunogenicity of ES-derived cells must be addressed and compared with allogenic epidermal cells to demonstrate any advantage over adult somatic cells.

Similar to all kinds of allogenic transplantation, the problem of immune rejection exists if and when ES-derived cells are used for therapeutics. As previously suggested, the current availability of a broad collection of human ES cell lines may be beneficial. Interestingly, it was recently demonstrated that human ES-derived cells might be much less immunogenic than

their adult counterparts [91]. Accordingly, it appears that the ES-derived ectodermal cell population that we described previously display low HLA1 and HLA2 at their surface, suggesting potential low immunity [77]. A great effort has to be made regarding the monitoring of tolerance and immunogenicity of the differentiated ES cells. Only then will we be able to tell whether ES cell technology may be an alternative cellular source for skin cell therapy.

Five-year view

As previously mentioned, the identification of epidermal and corneal stem cell markers is desirable for their efficient purification and retransplantation. In this sense, the elucidation of the molecular signaling and mechanisms that induce epidermal or corneal commitment and keratinocyte stem cell maintenance may contribute to therapeutic applications. Therefore, the value of ES cellular models that recapitulate embryonic lineage-specific development is unquestionable and they might identify new markers for somatic stem cell purification. In particular, this technology will enable further study of the functions of candidate genes and molecules that mediate the embryonic induction of these specific lineages and postnatal tissue maintenance. Furthermore, the ES cell technology will be undoubtedly useful as an alternative cellular model for toxicopharmacological tests and drug design, which could replace the use of animals and *in vivo* experiments. It will accommodate the new European guidelines.

A fundamental issue that must be highlighted is the possible functionality of those highly enriched ES-derived populations of epidermal or corneal cells to form tissue equivalents by organotypic reconstitution and to transplant them onto animal models. This kind of examination may reveal important information with regard to the quality of our ES-derived cells, in a more meaningful way than simple marker succession. The transplanted tissue must be compared with adult tissue for graft take, multipotency and repair. Furthermore, the issue of their immunogenicity must be assessed, and perhaps only then can we evaluate whether ES cells could be an alternative for tissue regeneration.

The recent identification of the precise pathways that induce de-differentiating somatic cells into ES-like cells is fascinating [92,93]. In these studies, the generation of induced pluripotent stem (iPS) cells was facilitated by retroviral transduction of the identical cassette of four transcription factors (Oct4, Sox-2, Klf-4/nanog and c-Myc/LIN-28) into adult or mouse [94,95] human [92,93] cells. This manipulation was also feasible without c-Myc [96]. Consequently, ES-like colonies of cells were established and shown to be reminiscent of ES cells by means of morphology, various expressed markers, differentiation capabilities and induction of teratoma. It is tempting to speculate or hope that, in the future, it will be possible to isolate somatic cells from a patient, de-differentiate them *ex vivo* into pluripotent ES cells and finally reprogram those cells into the desired cell lineage. Nonetheless, as the iPS cells contain numerous retroviral integration sites in their chromatin, their submission for treatment may result in cancer development. As the authors stated, 'Even with the presence of retroviral integration, human iPS cells are useful for understanding disease mechanisms, drug screening and toxicology'. Future studies must focus on alternative strategies for obtaining iPS cells by transient enhanced expression of these transcription factors without genetic alterations. The accumulating data regarding the differentiation of ES cells into a specific cellular fate, such as epidermal fate, will certainly be extremely valuable for completing this as yet only imaginaed practice of autotransplantation. The production of iPS cells from patient cells could be an exciting alternative for gene therapy since pluripotent cells are able, contrary to adult somatic cells, to undergo homologous recombination, which can enable repair of the mutation through a knock-in approach. For this reason, it would be absurd to oppose these two approaches, which ultimately may become complementary.

Key issues

- The skin and cornea are related tissues in terms of structure, function and developmental origin.
- The high tissue turnover of skin and cornea depends on similar keratinocyte stem cells that reside in segregated niches.
- For the purpose of skin and corneal therapy, it is better to purify the corresponding stem cells from a patient, to expand them *ex vivo*, repair them if required and finally retransplant them.
- A major challenge is to propagate adult epidermal stem cells *in vitro* in conditions that facilitate the maintenance of their complete pluripotency.
- In cases where there is a complete loss of stem cells, an immediate necessity for treatment or a deficiency in donor allografts, an alternative cellular source, such as human embryonic stem (ES) cell-derived precursors, may be required.
- Pluripotent human ES cells can serve as an unlimited cellular source that can be directed into many kinds of cellular lineages, including epidermal and corneal lineages.
- Homogeneous human ES-derived keratinocytes were successfully isolated but could hardly be amplified *in vitro*.
- As an alternative approach, earlier precursor cells that can be propagated in culture were derived from ES cells. These ectodermal precursors can give rise to keratinocytes (as well as to corneal cells) and must be further evaluated *in vivo*.

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