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ORIGINAL ARTICLE

Tissue engineering of skin: human tonsil-derived mesenchymal cells can function as dermal fibroblasts

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Abstract
Purpose It is unclear whether dermal fibroblasts are indispensable key players for tissue engineering of dermo-epidermal skin analogs. In this experimental study, we wanted to test the hypothesis that tonsil-derived mesenchymal cells can assume the role of dermal fibroblasts when culturing pigmented skin analogs for transplantation.
Methods Mesenchymal cells from excised tonsils and keratinocytes, melanocytes, and fibroblasts from skin biopsies were isolated, cultured, and expanded. Melanocytes and keratinocytes were seeded in a ratio of 1:5 onto collagen gels previously populated either with tonsil-derived mesenchymal cells or with autologous dermal fibroblasts. These laboratory engineered skin analogs were then transplanted onto full-thickness wounds of immunoincompetent rats and analyzed after 3 weeks with regard to macroscopic and microscopic epidermal characteristics.
Results The skin analogs containing tonsil-derived mesenchymal cells showed the same macroscopic appearance as the ones containing dermal fibroblasts. Histologically, features of epidermal stratification, pigmentation, and cornification were identical to those of the controls assembled with autologous dermal fibroblasts. Transmission electron microscopy confirmed these findings.
Conclusion These data suggest that human tonsil-derived mesenchymal cells can assume dermal fibroblast functions, indicating that possibly various types of mesenchymal cells can successfully be employed for "skingineering" purposes. This aspect may have clinical implications when sources for dermal fibroblasts are scarce.

Keywords Human-pigmented skin analog · Palatine tonsil · Mesenchymal cells · Mesenchymal–epithelial interaction · Epidermal stratification and cornification · Tissue engineering

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Introduction

Donor skin can be a substantial limiting factor for burn, plastic, and reconstructive surgeons when large full-thickness skin defects need to be covered. A promising development to overcome this limitation is the engineering of skin substitutes in several laboratories worldwide, which has made significant progress over the last few decades [1–3]. At first, only epidermal sheets, such as autologous cultured epidermal autografts (CEA), were used for coverage, but functional and esthetic results were unsatisfying because of inconsistent graft take, susceptibility to infection, and graft contracture [4–10]. With the addition of

dermal fibroblasts to skin substitutes, a more stable skin construct with better functional and esthetic results has been created [11–13]. Fibroblasts produce extracellular matrix components to strengthen the dermal compartment and interact with cells of the epidermal compartment including keratinocytes and melanocytes. This mesenchymal–epithelial interaction is crucial to regulate cell differentiation and proliferation, as well as to maintain tissue homeostasis of the epidermis [14].

It is unclear, whether autochthonous dermal fibroblasts are in fact indispensable key players to fulfill the various roles of support and interaction in tissue-engineered skin analogs or whether other mesenchymal cells could substitute the functions exerted by fibroblasts. In this experimental study, we tested the hypothesis whether palatine tonsil-derived mesenchymal cells, as a possible alternative cell source if dermal fibroblasts are scarce, can competently substitute for dermal fibroblasts in tissue-engineered pigmented skin analogs. Pigmented dermo-epidermal skin analogs with tonsil-derived mesenchymal cells were tested in a rat model and features of epidermal stratification, cornification, and pigmentation were compared to pigmented skin analogs entirely derived from human skin biopsies.

Materials and methods

Human skin and tonsil samples

Human foreskins and palatine tonsils were collected after the parents gave informed consent to use the samples. Human foreskins were obtained after circumcision from patients 1–16 years of age and were kept in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Basel, Switzerland) until processing. Palatine tonsils were obtained from children aged 3–9 years undergoing tonsillectomy for recurrent tonsillitis or hyperplasia and were postoperatively processed as described by Giger et al. [15]. Foreskins and tonsils were used for cell isolation as described below; for histological examination, they were embedded in O.C.T.^{TM} compound (Sakura Finetek, Alphen aan den Rijn, the Netherlands) and kept at −20 °C, or prepared for paraffin sections. The study was conducted according to the Declaration of Helsinki Principles and after permission by the Ethics Commission of the Canton Zurich.

Isolation and culturing of primary cells

Human epidermal keratinocytes, melanocytes, and dermal fibroblasts were extracted from foreskins. The method used for keratinocyte and fibroblast isolation, culture, and expansion is described in detail by Schneider et al. [16]; the method for melanocyte processing is specified by Böttcher-Haberzeth et al. [17].

Mesenchymal cells were extracted from human palatine tonsils. First, tonsils were rinsed in 70 % ethanol for 30 s, then they were incubated overnight at 4 °C in 2.5 ml dispase (BD Falcon, Heidelberg, Germany) and 2.5 ml HBSS (Gibco Life Technologies, Zug, Switzerland) containing 1,500 U/ml Penicillin and 1.5 mg/ml Streptomycin (Gibco Life Technologies, Zug, Switzerland), 1.5 mg/ml Gentamycin (Gibco Life Technologies, Zug, Switzerland), and 3.5 µg/ml Fungizone (Gibco Life Technologies, Zug, Switzerland). The epithelial part of the tonsils was peeled off and fibroblasts were isolated and cultured from the mesenchymal tissue as described in Biedermann et al. [18]. To test the possibility of isolating mesenchymal cells from a small tissue sample, cells were also isolated according to the above-described method but from a 5-mm punch biopsy.

Preparation of tissue-engineered skin analogs

The tissue-engineered skin analogs were prepared according to the protocol described by Pontiggia et al. [19]. Shortly, human tonsil-derived mesenchymal cells (1×10^5, passage 1) or human dermal fibroblasts (1×10^5, passage 1) were mixed with rat collagen type I and filled in cell culture inserts (all BD Falcon, Germany). Then, DMEM (Invitrogen, Switzerland) was added to the dermal equivalents. After 6 days of cultivation, 5×10^5 melanocytes and keratinocytes (passage 1) were seeded in a ratio of 1:5 on top of both types of dermal equivalents. The skin analogs were then cultured for another week in a 1:5 mixture of melanocyte growth medium (Promocell, Heidelberg, Germany) and keratinocyte medium (SFM, Invitrogen, Basel, Switzerland) and thereafter transplanted.

Transplantation of tissue-engineered skin analogs

The Local Committee for Experimental Animal Research approved the study protocol (permission number 76/2011). The surgical procedure was performed as described previously by Biedermann et al. [20]. In short, the above-described tissue-engineered skin analogs containing human tonsil-derived mesenchymal cells ($n = 4$) or human dermal fibroblasts ($n = 4$) were transplanted onto full-thickness skin defects on the back of immuno-incompetent nu/nu rats (female 8–10 weeks old, Harlan Laboratories, Netherlands), previously prepared and anesthetized as specified by Böttcher-Haberzeth et al. [21]. To ensure protection of the skin analogs and to prevent wound closure from the surrounding rat skin, steel rings (26 mm in diameter) were sutured to the rat skin with non-absorbable polyester

sutures (Ethibond, Ethicon, USA) prior to application of the analogs. The skin analogs were then covered with a multilayer wound dressing consisting of a silicone foil (Silon-SES, BMS, Allentown, USA), a polyurethane sponge (Ligasano, Ligamed, Ötztal, Austria), a cohesive conforming bandage (Sincohaft, Theo Frey AG, Switzerland), and tape. Dressing changes and photographs of the transplants were performed weekly. After 3 weeks, the transplanted skin analogs were excised, processed (for cryosections, paraffin sections, and electron microscopy), and analyzed.

Histological analysis and immunohistochemical staining

Paraffin sections (5 μm) were deparaffinized in xylene, rehydrated, and stained with hematoxylin and eosin (Sigma, Buchs, Switzerland), with the Fontana Masson technique, and with an antibody staining against microphthalmia-associated transcription factor [MITF (SPM 290:sc-56433, Santa Cruz, Nunningen, Switzerland)] to assess morphology, melanin distribution, and melanocytes distribution.

Cryosections (10 μm) were used for immunofluorescence staining as previously described by Pontiggia et al. [22]. Different antibodies were used according to the manufacturer's description to visualize melanosomes [HMB45 (clone Hmb-45, 1:50, Dako, Switzerland)], the basement membrane [Lam5 (clone P3H9-2, 1:100, Santa Cruz, Switzerland)], components of epithelial cells [CK1 (clone LHK1, 1:200; Chemicon, Switzerland), CK10 (clone DE-K10; 1:100, Dako, Switzerland), Loricrin (clone ab2472, 1:500, Abcam, Switzerland), Occludin (polyclonal, 1:50, Zymed, Invitrogen, Switzerland), CK15 (clone spm190, 1:50; Santa Cruz, Switzerland), CK19 (clone RCK108 1:50, Dako, Switzerland), E-cadherin (clone 36/E-cadherin, 1:50, BD Pharmingen, Switzerland), CK16 (clone LL025, 1:100; Chemicon, Switzerland)], and human fibroblasts [CD90 (clone 5E10, 1:50, Dianova, Germany)]. As a secondary antibody, we used TRITC- and FITC-conjugated polyclonal goat F(ab')2 fragments directed to mouse or rabbit immunoglobulins (Dako, Baar, Switzerland). Pictures of immunofluorescence staining were taken with a DXM1200F digital camera connected to a Nikon Eclipse TE2000-U inverted microscope. The device is equipped with Hoechst 33342-, FITC-, and TRITC-filter sets (Nikon AG, Switzerland; Software: Nikon ACT-1 vers. 2.70). Images were processed with Photoshop 7.0 (Adobe Systems Inc, Germany).

Chromameter measurements

Immediately before excision of the transplants, the color of the skin analogs was measured using a Chromameter CR 400 (Konica Minolta, Osaka, Japan), which defines a specific color in a three-dimensional color space ($L^*a^*b^*$). For analysis of the visible spectrum of light reflected from the different skin analogs, only the L value (mean ± SD) of the reflectance spectroscopy was processed, as it is the most sensitive of the trichromatic values to skin analog color change [23]. The L value correlates to perceived lightness and it can range from absolute black (0) to absolute white (+100).

Statistical analysis

The L values of the different skin analogs were recorded. All results are shown as mean ± standard deviation (SD). Statistical analysis was performed with Excel 2013 (Microsoft Corporation, Ontario, Canada). Comparison between two groups was performed using the unpaired student's t test. Results were considered significant with a $p < 0.001$.

Electron microscopy

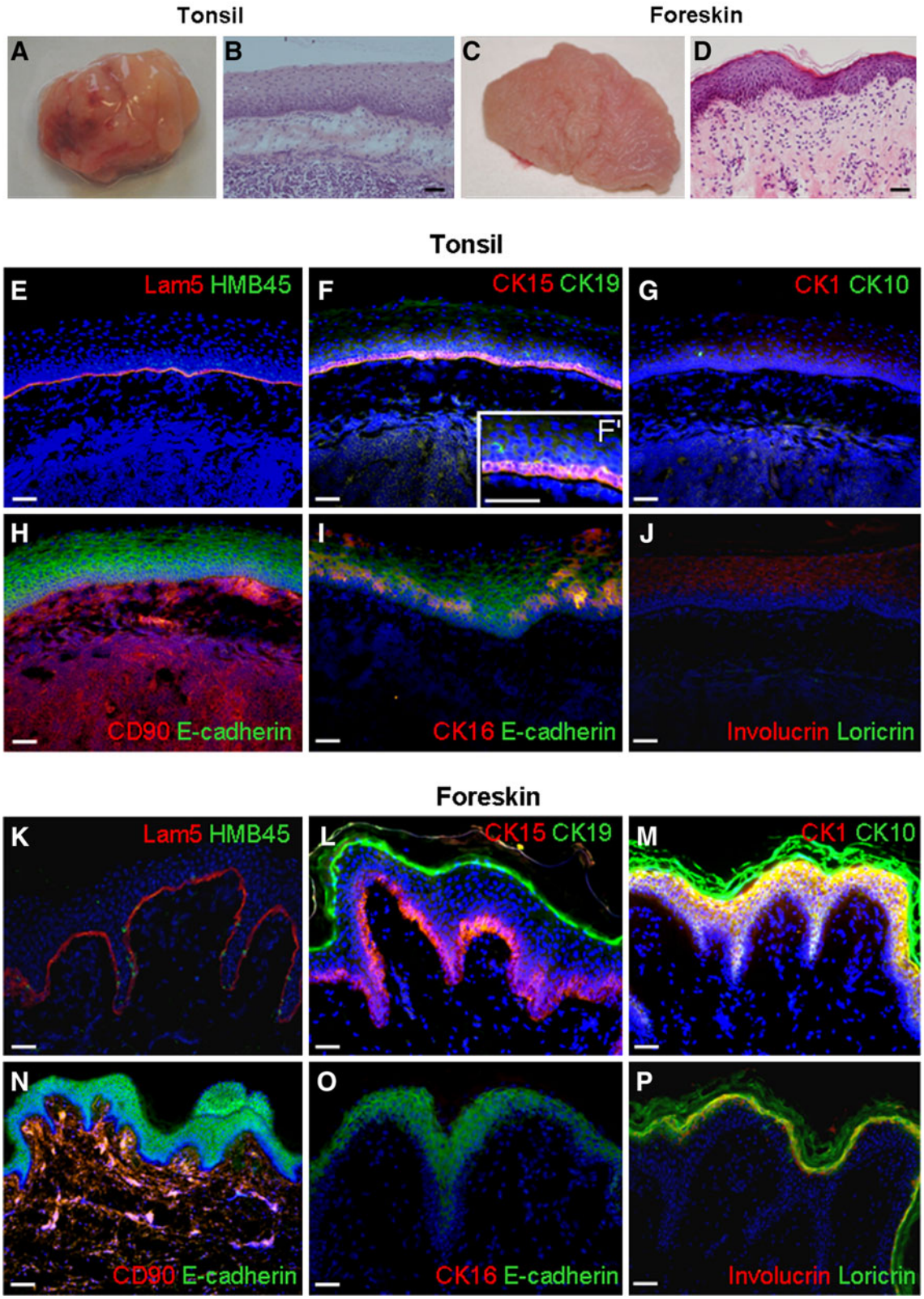
Tissue blocs of 1 mm^3 were prefixed in 0.1 M cacodylate buffer (Merck, Germany), pH 7.3 containing 2.5 % glutaraldehyde for 2 h, then washed in cacodylate buffer, postfixed with an aqueous solution of 1 % OsO_4 and 1.5 % $K_4Fe(CN)_6$ for 1 h, dehydrated, and finally embedded in EPON 812 (Catalys AG, Switzerland) for transmission electron microscopy analysis. From these blocs, ultrathin sections of 50–70 nm were collected on copper grids, contrasted with 4 % uranyl acetate and 3 % lead citrate, and examined with a CM 100 transmission electron microscope (Philips, the Netherlands). All reagents used were from Sigma unless mentioned otherwise.

Results

Histological analyses of native human palatine tonsils and foreskins

The mucous human palatine tonsil (Fig. 1a) and the non-mucous human foreskin (Fig. 1c), although very different in embryologic origin and macroscopic aspect, have a similar basic structure. Both are composed of multilayered epithelium and underlying mesenchymal tissue (Fig. 1b, d).

The various differences in marker expression patterns between tonsils and foreskins are summarized in Table 1 and shown in Fig. 1e–p. Taken together, both have a multilayered epithelium, but show a different expression pattern of stratification and cornification markers, and, most importantly, the epithelium of palatine tonsils does not contain melanocytes.


Tonsil
Foreskin
A
B
C
D
Tonsil
E
Lam5 HMB45
F
CK15 CK19
F'
G
CK1 CK10
H
CD90 E-cadherin
I
CK16 E-cadherin
J
Involucrin Loricrin
Foreskin
K
Lam5 HMB45
L
CK15 CK19
M
CK1 CK10
N
CD90 E-cadherin
O
CK16 E-cadherin
P
Involucrin Loricrin

◀ **Fig. 1** Macroscopic view and histological analysis of human palatine tonsils and human foreskins. **a** Macroscopically, the palatine tonsil shows a moist mucus surface. **c** In contrast, the foreskin shows a dry surface, typical for a keratinized and cornified epithelium. **b**, **d** Microscopically, the tonsil and the foreskin both show a multilayered epithelium with a cellularized mesenchyme, respectively dermis. A stratum corneum can only be seen in the foreskin. **e**–**j** Immunofluorescence staining showing the expression of epithelial structural markers in the palatine tonsil and **k**–**p** in the foreskin. Antibodies against the following components were applied to cryosections: **e**, **k** laminin 5 (*Lam5, red*) and HMB45 (*green*); **f**, **l** cytokeratin 15 (*CK15, red*) and cytokeratin 19 (*CK19, green*); **f′** shows a higher magnification of the basal layer of the tonsil; **g**, **m** cytokeratin 1 (*CK1, red*) and cytokeratin 10 (*CK10, green*); **h**, **n** CD90 (*red*) and E-cadherin (*green*); **i**, **o** cytokeratin 16 (*CK16, red*) and E-cadherin (*green*); **j**, **p** involucrin (*red*) and loricrin (*green*). *Scale bar* for all *panels* 50 μm

Table 1 Comparison of epithelial and mesenchymal/dermal marker expression pattern in palatine tonsils and in the foreskin

Markers	Expression in palatine tonsil	Expression in foreskin
Laminin 5	Consistent expression underneath multilayered epithelium (basement membrane present)	Consistent expression underneath multilayered epithelium (basement membrane present)
HMB45	No expression	Expression in basal layer (melanocytes present)
CK15/19	Uniform expression of both in basal layer (superimposed)	CK15: Uniform expression in basal layer; CK19: No expression (this pattern combined sign of epithelial homeostasis and maturation)
CK1/10	No expression	Uniform and superimposed expression in suprabasal layers (sign of stratification)
E-cadherin	Expression in all epithelial cells	Expression in all epithelial cells
CD90	Expression in mesenchyme (mesenchymal cells)	Expression in dermis (mesenchymal cells, mainly fibroblasts)
CK16	Scattered expression	No expression (wound marker)
Loricrin	No expression	Expression in the uppermost layers of epidermis (sign of cornification)
Involucrin	Diffuse expression in suprabasal layers of the epithelium	Expression in the uppermost layers of epidermis (sign of cornification)

Analysis of transplanted skin analogs

Three weeks after transplantation, the skin analogs built with tonsil-derived fibroblasts (Fig. 2a) showed the same macroscopic appearance as the tissue-engineered skin constructed with dermal fibroblasts (Fig. 2b) regarding structure and color. Compatible with this, microscopically both constructs showed a multilayered, stratified epidermis with a distinct stratum corneum and a loose, cellular dermal compartment (Fig. 2c, i).

A more in-depth investigation of specific epithelial markers revealed very similar expression patterns in both skin analogs. Transplants prepared with tonsil-derived mesenchymal cells showed a uniform expression of CK15 in the basal layer with subgroups of CK19-positive cells (Fig. 2d); a CK1 and CK10 superimposed expression in all suprabasal layers (Fig. 2e); an even E-cadherin expression in all epidermal cells and human CD90-positive cells in the dermal compartment (Fig. 2f); a patchy expression of CK16 throughout the suprabasal layers of the epidermis (Fig. 2g); an expression of both markers of cornification, loricrin and involucrin, with a detectable gradual shifting to the upper layers of the epidermis (Fig. 2h).

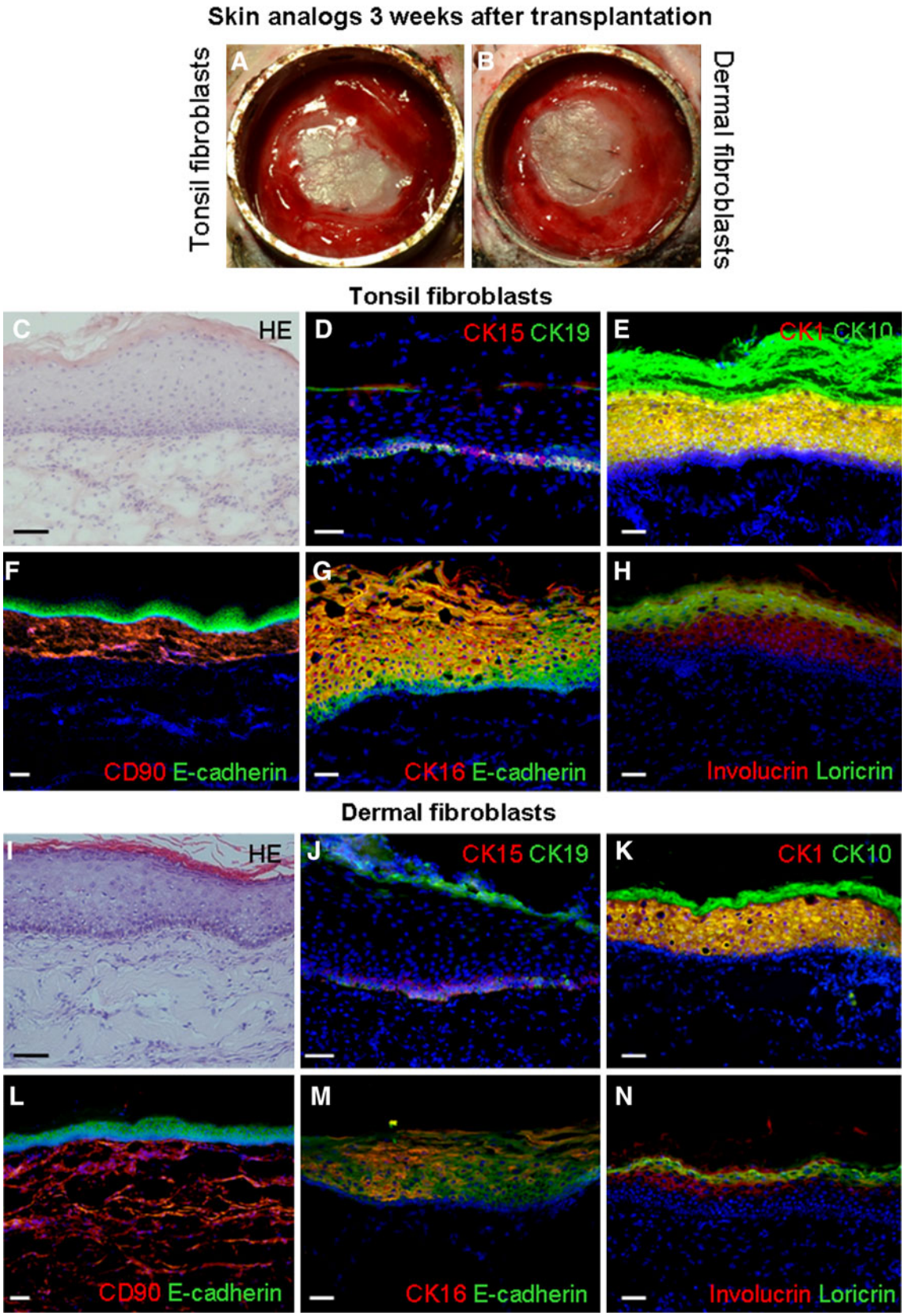
The only identifiable differences in the transplants constructed with dermal fibroblasts (Fig. 2j–n) were a less pronounced CK16 expression of the keratinocytes and a more advanced shift of the cornification marker to the upper epidermal layers.

Thus, after transplantation, skin analogs from both origins show a multilayered, stratified, and cornified epithelium with expression of stratification and cornification markers.

Melanocytes and melanogenesis in transplanted skin analogs

Using chromameter evaluation, we objectivized the macroscopically similar color of both transplants (Fig. 3a). We recorded no clear color difference between tonsil-derived skin (mean L value 53.94 ± 1.1 SD) versus foreskin-derived skin (mean L value 52.27 ± 0.9 SD), indicating a similar melanin production by melanocytes and a similar distribution to the keratinocytes within these constructs. In contrast, a statistically significant color difference was measured in skin analogs produced with dermal fibroblasts, but without melanocytes (mean L value 67.04 ± 1.67 SD; $p < 0.001$).

Immunofluorescence staining of melanosomes (HMB45) showed melanocytes in contact with the basement membrane and dendrites projecting to the upper layers of the epidermis (Fig. 3b, c) in both constructs. An immunohistochemical analysis of MITF expression, a nuclear melanocyte marker, showed evenly distributed melanocytes in a physiological position, i.e. in the basal layer, of the epidermis in both analogs (Fig. 3d, e). A typical supranuclear melanin distribution within the epidermal cells could be demonstrated with a Fontana Masson staining, highlighting melanin in melanocytes and keratinocytes (Fig. 3f, g).


Skin analogs 3 weeks after transplantation
Tonsil fibroblasts
Dermal fibroblasts
A
B
Tonsil fibroblasts
C
HE
D
CK15 CK19
E
CK1 CK10
F
CD90 E-cadherin
G
CK16 E-cadherin
H
Involucrin Loricrin
Dermal fibroblasts
I
HE
J
CK15 CK19
K
CK1 CK10
L
CD90 E-cadherin
M
CK16 E-cadherin
N
Involucrin Loricrin

◀ **Fig. 2** Macroscopic view and histological analysis of pigmented skin analogs 3 weeks after transplantation. **a** Macroscopically, the skin analog constructed with tonsil-derived mesenchymal cells in the dermal compartment shows a similar aspect to **b** the skin analog constructed with dermal fibroblasts. **c**, **i** Microscopically, the epidermis of both analogs shows a multilayered, stratified, and cornified epithelium with an underlying dense dermis. **d**–**h** Immunofluorescence staining showing the expression pattern of epithelial markers of stratification and cornification in skin analogs with tonsil-derived mesenchymal cells and **j**–**n** in skin analogs with dermal fibroblasts. Antibodies against the following components were applied to cryosections: **d**, **j** cytokeratin 15 (*red*) and cytokeratin 19 (*green*); **e**, **k** cytokeratin 1 (*red*) and cytokeratin 10 (*green*); **f**, **l** CD90 (*red*) and E-cadherin (*green*); **g**, **m** cytokeratin 16 (*red*) and E-cadherin (*green*); **h**, **n** involucrin (*red*) and loricrin (*green*). *Scale bar* for all *panels* 50 μm

These findings were confirmed by transmission electron microscopy (TEM) in the tonsil-derived skin analogs (Fig. 4). Melanocytes containing melanosomes were detected in the basal layer of the epidermis in close contact with the basement membrane and with fibroblasts in the dermal compartment. Surrounding keratinocytes, distinguished by the presence of desmosomes, showed melanin incorporated in a normal supranuclear position.

Thus, skin analogs constructed with mesenchymal cells from palatine tonsils and from foreskins show an integration and function of melanocytes within the epithelium, reflected by a similar color of both transplants.

Discussion

This is the first report providing evidence that palatine tonsil-derived mesenchymal cells can assume functions of dermal fibroblasts in a tissue-engineered pigmented dermo-epidermal skin analog. In particular, we showed that tonsil-derived mesenchymal cells could support the process of epidermal stratification, cornification, and pigmentation after transplantation almost identically as dermal fibroblasts do. The following aspects deserve to be addressed in more detail.

Although human palatine tonsils and foreskins have an entirely different embryologic origin with tonsils arising from an endodermal and foreskins from an ectodermal epithelium [24, 25], the tectonic structure and marker expression patterns of their epithelium surprisingly show many similarities. Both consist of a multilayered, stratified epithelium and an underlying loose mesenchymal/dermal tissue and both show a similar expression of certain cytokeratins and other epithelial markers [21, 26, 27]. But, some crucial differences can be seen between the two epithelia: the palatine tonsil epithelium does not show distinct signs of differentiation or stratification (missing expression of CK1 and CK10), it does not have an epithelium in homeostasis as an interfollicular epidermis does (persistent expression of CK19 in all basal cells), it does not show a developed cornified protective layer (lacking an organized expression of loricrin and involucrin), and, especially, there are no melanocytes present in their epithelium.

The importance of fibroblasts influencing and supporting a specific epithelium and maintaining its homeostasis [14, 28, 29] is well documented. Therefore, it is even more surprising that tonsil-derived mesenchymal cells (present in transplanted skin substitutes demonstrated by a human CD90 staining), which usually direct their adjacent epithelium to develop into a mucous, non-cornified cover, can take over the role of dermal fibroblasts in a tissue-engineered skin substitute and support a correctly stratified and cornified epithelium formation after transplantation. The anatomical structures as well as the expression patterns of multiple epidermal markers of skin analogs with tonsil-derived mesenchymal cells and of those with dermal fibroblasts show practically no difference. Both exhibit a multilayered, stratified epidermis with a thick stratum corneum, similar to that of normal interfollicular epidermis. Markers of differentiation, maturation, and stratification (CK15, CK19, CK1, CK10) are similarly expressed as expected 3 weeks after transplantation. The same holds true for markers indicating a developing cornified layer (involucrin, loricrin). Only the epidermal wound marker CK16 shows a more pronounced expression in the skin analog constructed with tonsil-derived mesenchymal cells. Possibly, this stronger expression results from the fact that normal tonsil epithelia already contain CK16-positive cells (Fig. 1i) and that the "new" type of mesenchymal–epithelial interaction triggers additional CK16 expression. Alternatively, the epithelium is slower in reaching a homeostatic state after transplantation and remains longer in a wound healing mode, characterized by high CK16 expression, due to the tonsil-derived mesenchymal cells, which are new to this business. Interestingly, a similar delay phenomenon was observed when non-professional keratinocytes, namely sweat gland-derived keratinocytes, were used to engineer skin [17].

Another remarkable finding is the support of a physiological, epidermal melanocyte position, function, and melanin distribution in the skin analogs constructed with tonsil-derived mesenchymal cells. The physiological melanocyte function, in particular melanin production and deposition to achieve constitutive skin pigmentation, requires a correct cell–cell interaction between mesenchyme and epithelium [14, 30, 31]. For instance, it was shown that by using keratinocytes, melanocytes, and fibroblasts from the same donor skin to engineer pigmented dermo-epidermal skin analogs, the original donor skin color can be reproduced, regardless of which concentration

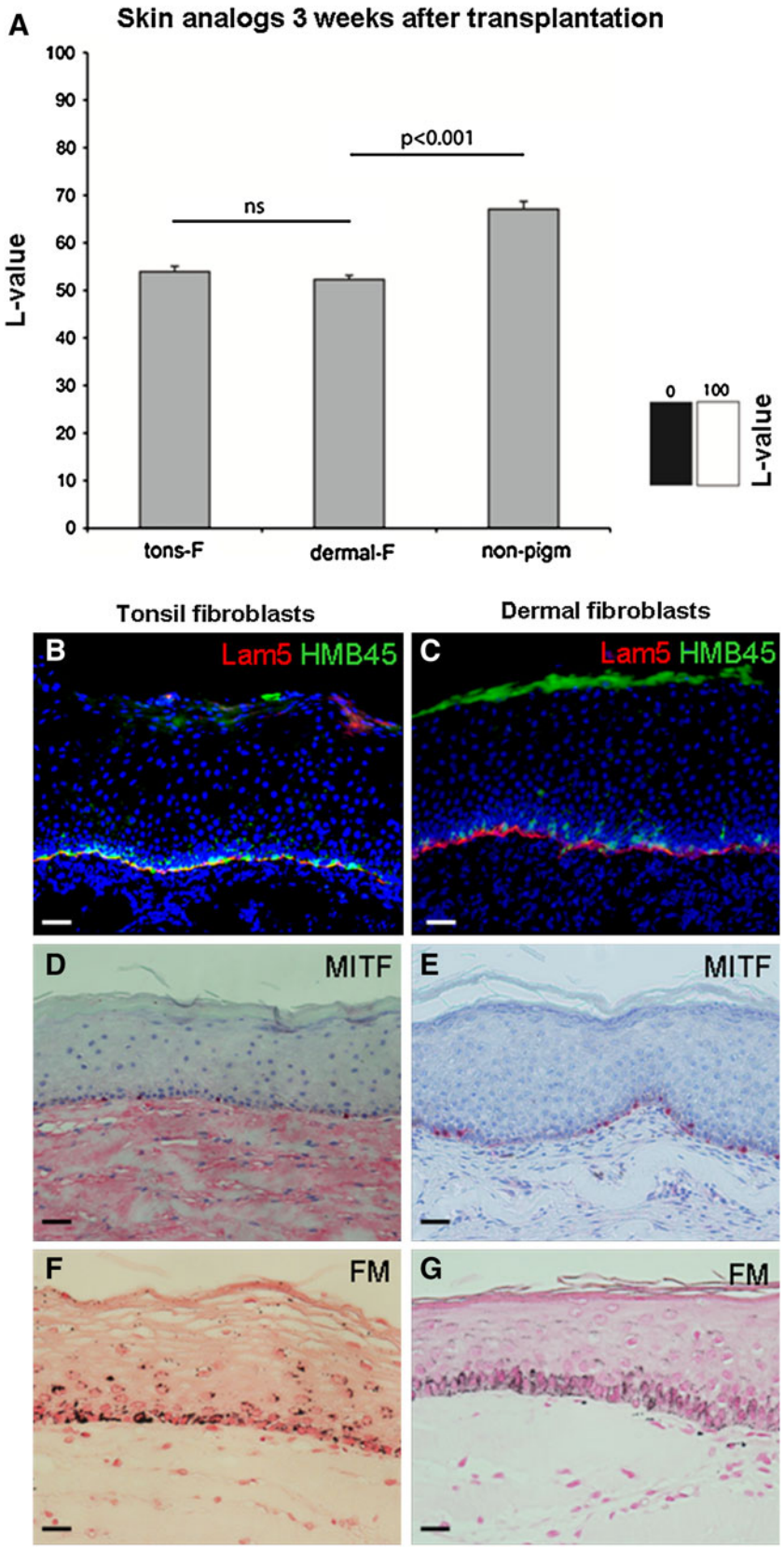


Fig. 3 Melanocyte function and position in pigmented skin analogs constructed with tonsil-derived mesenchymal cells and with dermal fibroblasts 3 weeks after transplantation. **a** Chromameter evaluation (mean *L* value of all transplants, 0 = *black*, 100 = *white*) of pigmented skin analogs with tonsil-derived fibroblasts (tons-F) and with dermal fibroblasts (dermal-F) and of skin analogs without addition of melanocytes (non-pigm). *ns* Non significant. **b**, **d**, **f** Specific staining of melanosomes, melanocytes, and melanin in pigmented skin analogs with tonsil-derived fibroblasts and **c**, **e**, **g** with dermal fibroblasts. **b**, **c** Immunofluorescence staining with antibodies against a component of the basement membrane (Laminin 5, *red*) and against melanosomes (HMB45, *green*). **d**, **e** Immunohistochemical staining with an antibody against MITF, a nuclear staining of melanocytes (*red*). **f**, **g** Fontana-Masson (FM) staining highlighting the melanin distribution in the epidermis (melanin stains *black*). *Scale bar* for all *panels* 10 μm

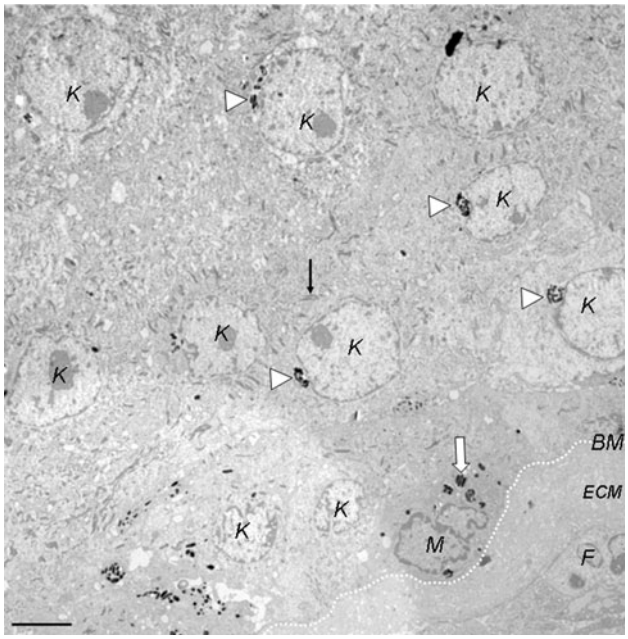


Fig. 4 Ultrastructural evaluation (TEM) of a pigmented skin analog with tonsil-derived mesenchymal cells 3 weeks after transplantation. Melanocyte (*M*), containing melanosomes (*white arrow*) are in contact with the basement membrane (*BM*, *dotted line*) and in vicinity to fibroblasts (*F*) and their surrounding extracellular matrix (*ECM*). In the upper layers of the epidermis, keratinocytes (*K*), connected to one another by desmosomes (*black arrow*), contain melanin (*white arrowheads*) in a supranuclear position. *Scale bar* 5 μm

of melanocytes and keratinocytes was applied, underscoring the cybernetic power of fibroblasts [23]. In contrast, if keratinocytes, melanocytes, and fibroblasts from different anatomical regions or from different skin pigmentation types are used to construct skin analogs, various melanocyte activities and, consequently, different skin colors are obtained [28, 29, 32].

In the present experimental study, melanocytes remained in the epidermis, found a physiological position in the stratum basale, and showed a continuous melanin production with correct supranuclear melanin incorporation into keratinocytes. Importantly, the amount of melanin deposited was apparently similar to that of controls as reflected by similar color of both types of skin analogs.

In conclusion, this is, to the best of our knowledge, the first study demonstrating that palatine tonsil-derived mesenchymal cells can be used instead of dermal fibroblasts to engineer and successfully transplant pigmented dermo-epidermal skin analogs. Apparently, these mesenchymal cells can fully assume many key roles that dermal fibroblasts usually play in this tissue engineering context. This unexpected phenomenon indicates that possibly various other types of mesenchymal cells can successfully be employed for skingineering or other tissue engineering purposes. As a sufficient amount of fibroblasts to prepare a pigmented dermo-epidermal skin analog can be extracted from a tonsil biopsy as small as 5 mm in diameter, this aspect might, exceptionally, even have clinical implications when sources for dermal fibroblasts are scarce, such as in extremely large burns, or when dermal fibroblasts are pathological and cannot be used for tissue engineering.

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Conflict of interest The authors declare that they have no conflict of interest.

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