Pediatr Surg Int (2014) 30:1241–1247
DOI 10.1007/s00383-014-3614-7

ORIGINAL ARTICLE

Experimental tissue engineering of fetal skin

L. Mazzone · L. Pontiggia · E. Reichmann · N. Ochsenbein-Kölble · U. Moehrlen · M. Meuli

Accepted: 7 October 2014 / Published online: 22 October 2014

Abstract
Purpose In some human fetuses undergoing prenatal spina bifida repair, the skin defect is too large for primary closure. The aim of this study was to engineer an autologous fetal skin analogue suitable for in utero skin reconstruction during spina bifida repair.
Methods Keratinocytes (KC) and fibroblasts (FB) isolated from skin biopsies of 90-day-old sheep fetuses were cultured. Thereafter, plastically compressed collagen hydrogels and fibrin gels containing FB were prepared. KC were seeded onto these dermal constructs and allowed to proliferate using different culture media. Constructs were analyzed histologically and by immunohistochemistry and compared to normal ovine fetal skin.
Results Development of a stratified epidermis covering the entire surface of the collagen gel was observed. The number of KC layers and degree of organization was dependent on the cell culture media used. The collagen hydrogels exhibited a strong tendency to shrink after eight to ten days of culture in vitro. On fibrin gels, we did not observe the formation of a physiologically organized epidermis.
Conclusion Collagen-gel-based ovine fetal cell-derived skin analogues with near normal anatomy can be engineered in vitro and may be suitable for autologous fetal transplantation.

Keywords Fetus · Spina bifida · Skin · Tissue engineering · Fetal surgery

L. Mazzone (✉) · U. Moehrlen · M. Meuli
Department of Surgery, University Children's Hospital Zurich, Zurich, Switzerland
e-mail: luca.mazzone@kispi.uzh.ch

L. Mazzone · N. Ochsenbein-Kölble · U. Moehrlen · M. Meuli
Center for Fetal Diagnosis and Treatment Zurich, Zurich, Switzerland

L. Mazzone · L. Pontiggia · E. Reichmann · M. Meuli
Tissue Biology Research Unit, Department of Surgery, University Children's Hospital Zurich, Zurich, Switzerland

L. Mazzone · L. Pontiggia · E. Reichmann · U. Moehrlen · M. Meuli
Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland

N. Ochsenbein-Kölble
Department of Obstetrics, University Hospital Zurich, Zurich, Switzerland

Introduction

Some congenital malformations go along with tissue defects. Where the lack of tissue does not allow for primary reconstruction, surgical correction becomes a true challenge. Large spina bifida lesions definitely belong to this category. Spina bifida, a severe congenital malformation affecting 1–2/1,000 life births, is characterized by an extrusion of spinal cord and meninges through an open defect of the spine, the adjacent soft tissue, and the skin. The standard surgical repair of the defect ideally consists of neural placode tubularization, dura closure, reinforcement of the dura closure site with paraspinal myofascial flaps, and finally skin closure. For many years, closure of the defect has been performed shortly after birth. In cases of large defects, rotational flaps, relief cuts, and skin transplantations have been used to achieve soft tissue coverage.

Since publication of clear-cut benefits of prenatal versus postnatal spina bifida repair by open fetal surgery (MOMS Trial [1]), open in utero repair has become a new treatment

option. Large defects during fetal repair, however, pose an additional challenge, as tissue friability does not allow skin closure under tension, and rotational flaps as used postnatally are considered too difficult and risky in the setting of fetal surgery.

Hypothetically, an autologous, tissue-engineered fetal dermo-epidermal skin substitute (fDESS) represents an ideal approach to obtain a functional, watertight, and definitive closure of the skin defect that, advantageously, also enhances neural tissue protection.

The aim of this study was, therefore, the experimental engineering of an autologous fetal skin analogue with the prospect of a possible in utero application for skin reconstruction during spina bifida repair.

Materials and methods

Cell cultures

For these experiments, stocks of cryopreserved fetal sheep KC and FB were used. These cells had been isolated from skin biopsies obtained in other experiments from timemated 90-day-old sheep fetuses (experiments approved by the local authority of animal welfare). Prior to cryopreservation, isolation had been performed as follows: The skin biopsies were stored in DMEM (Gibco, Basel, Switzerland) containing gentamycin 15 mg/ml (Gibco), Penicillin/Streptomycin 300 U/ml, respectively, 300 µg/ml (Sigma, Buchs, Switzerland), and Fungizone 250 ng/ml (Gibco) at 4 °C before being processed in the first 24 h after harvesting. For cell isolation, pieces were incubated for 1–2 h at 37° in 25 U/ml dispase (BD Biosciences, Allschwil, Switzerland) in Ham's F12 (Gibco) containing 5 ug/ml gentamycin. The epidermis was separated mechanically under the microscope. Thereafter, the epidermis was digested in prewarmed 0.5 % Trypsin/EDTA 10x (Gibco) for 2 min at 37 °C to isolate KC. These were suspended in CnT-57 keratinocyte medium (CELLnTEC Advanced Cell Systems AG, Bern, Switzerland) containing 5 µg/ml gentamycin, then seeded in collagen type I-coated (calf hide collagen from Symanthes, France) cell culture flasks (TRP, Switzerland), expanded to confluence and then cryopreserved in CnT-57 with 10 % DMSO (Sigma). The dermis was incubated for about 45 min at 37°, 5 % CO_2, in 2 mg/ml collagenase blend (Sigma), the hereby isolated FB were resuspended in DMEM medium supplemented with 10 % fetal calf serum (FCS), 4 mM L-alanyl-L-glutamine, 1 mM sodium pyruvate, and 5 ug/ml gentamycin, seeded in cell culture flasks and expanded. Cells were then cryopreserved in supplemented DMEM with 10 % DMSO. After unthawing, KC were seeded and expanded in collagen-I-coated flasks in CnT-57 containing 5 ug/ml gentamycin, FB in culture flasks in supplemented DMEM.

Organotypic cultures with different media for in vitro analysis

Collagen-based and fibrin-based dermo-epidermal skin substitutes were prepared using a transwell system (4.2 cm^2 six-well cell culture inserts with membranes of 3.0 µm pore size from BD Falcon, Basel, Switzerland).

A first series of collagen-based gel were prepared as follows: Per gel, 0.6 ml of chilled neutralization buffer (containing 0.15 M NaOH, 0.32 M $NaHCO_3$, 200 mM Hepes), 0.4 ml of supplemented DMEM/10 % FCS containing 50,000 of the cultured fetal sheep FB, and 2 ml calf hide collagen I (Symanthes) were mixed and poured in the insert. After approximately 4 h, the gels were compressed as previously described by Braziulis [2]. These gels were then cultured submersed in DMEM/10 % FCS for 7 days before KC (200,000–400,000 per gel) were seeded on the surface of each gel within siliconized polypropylene rings of 15 mm diameter to avoid dispersion. The ring was removed 4 h later. After the seeding, medium in the upper and lower compartment of the transwell system was changed to either CnT-09, CnT-FTAL-D (both from CELLnTEC) or to modified Rheinwald and Green medium (RGM; three parts of DMEM and one part of Ham's F12, 5 mg/ml gentamycin, 1.4 mM CaCl2, 0.4 mg/ml hydrocortisone, 5 mg/ml 1 insulin, 2 nM triiodothyronine, 180 mM adenine, 10 ng ml/1 EGF (all from Invitrogen, Basel, Switzerland), 0.1 nM choleratoxin [Calbiochem/VWR International AG, Dietikon, Switzerland) and 10 % lamb serum (LS) (from Gibco)]. Medium changes were performed every 2–3 days. Eight days after KC seeding, the gels were embedded for histological analysis.

For a second series of gels, slight modifications were done. In an attempt to counteract previously observed contraction of collagen-based gels, the gels of these series were combined with a reinforcement layer composed of Integra™ (Integra LifeScience, Plainsboro, NJ, USA) dermal template. The template was bathed in DMEM/10 % LS and the attached silicon membrane was detached mechanically. 500,000 FB suspended in DMEM/10 % lamb serum were sucked in the dermal template using a sterile filter flask under suction. Then, the Integra™ dermal template was placed on the membrane of the transwell insert, the collagen-gel solutions was poured over it, and hours later, the construct was compressed. In contrast to the first series of gels, the submersion media used here before KC seeding (400,000 per gel) was DMEM supplemented with 10 % LS instead of FCS and after seeding, DMEM/10 % LS was used in the lower compartment and CnT-09 in the upper compartment.

Fibrin-based gels were produced with a volume of 1.5 ml. Fibrinogen from bovine plasma (Sigma) was reconstituted in prewarmed NaCl to a final concentration of 10 mg/ml. 100,000 FB in DMEM were added to the filtered solution and hold on ice. Then, 11 µl/ml Thrombin (Sigma, 100 U/ml) was added, and the mixture was quickly poured in the insert. The gels were cultured submersed in DMEM/10 % LS until KC seeding (200,000 per gel) and thereafter in DMEM/10 % LS in the lower compartment and CnT-09 in the upper compartment.

Histology and immunofluorescence microscopy

The dermo-epidermal skin substitutes were embedded eight days after KC seeding in OCT compound (Sakura Finetek/Digitana AG, Horgen, Switzerland) and frozen to −20 °C. 10 µm cryosections of fDESS and of cryopreserved skin biopsies of 90 and 120 days gestational age (GA) were stained with hematoxylin and eosin (H&E, Sigma) and imaged by light microscopy.

10 µm sections were also used for immunofluorescence according to the protocol described by Pontiggia et al. [3]. Antibodies used to visualize components of epithelial cells were K5 (clone GP5-2, 1:100, Progen, Germany), K10 (clone DE-K10; 1:100, Dako, Switzerland), K14 (clone LL02, 1:50, Chemicon Switzerland), K15 (clone spm190, 1:50; Santa Cruz, Switzerland) and Loricrin (clone ab2472, 1:500, Abcam, Switzerland). Laminin 1&2 (clone ab7463, 1:500, Abcam) was used to visualize the basal membrane.

FITC-conjugated polyclonal immunoglobulins directed to mouse, guinea pig, and rabbit were used as a secondary antibodies, Hoechst 33342 (Sigma) for staining of nuclei.

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 NIS Elements 3.22.11). Images were processed with Adobe Photoshop Elements 11.0 (Adobe Systems Inc., Germany).

Results

Analysis of dermo-epidermal skin substitutes cultivated under different media

Development of an epidermal layer covering the entire surface of the collagen gel was observed under all conditions, but the number of KC layers and degree of organization was dependent on the media and the gel type used. Under CnT-09 alone, the epidermis appeared to be more regular and stratified, consisting of five to six intermediate layers, and a thin stratum corneum (Fig. 1a, b). Formation of a defined stratum basale was not completed yet eight days after KC seeding. This epidermis showed some similarity to fetal sheep skin at 90 days GA (Fig. 1g), particularly due to KC with large clear cytoplasma typically found in fetal skin. Under CnT-FTAL-D, a strong differentiation medium, the epidermis showed two to three layers with no stratification, but already signs of cornification (Fig. 1c). Under RGM/10 % LS, only one to two irregular and already cornified layers could be observed. Further, heaps of accumulated KC were spread over the whole surface (Fig. 1d). In the collagen-based gel where CnT-09

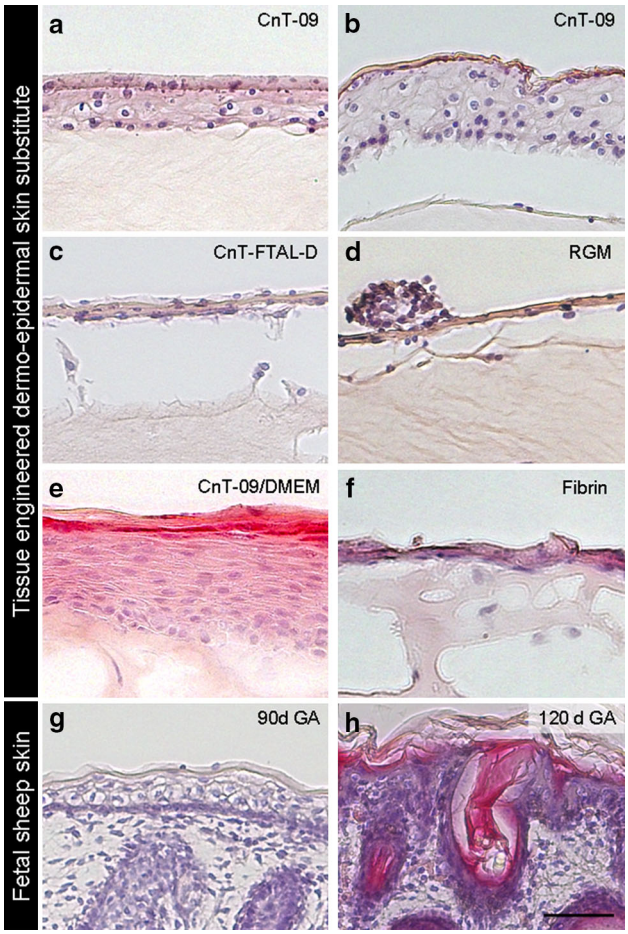

Fig. 1 H&E staining, panels with same magnification, *scale bar* 50 µm. **a**–**f** Histological results of in vitro tissue-engineered dermo-epidermal skin substitutes under different conditions; collagen-based gels: **a**, **b** two different images of the same substitute cultured with CnT-09, **c** substitute cultured with CnT-FTAL-D, **d** with supplemented RGM and **e** with CnT-09/supplemented DMEM. **f** Fibrin-based gel cultured with CnT-09/supplemented DMEM. **g**, **h** Depict fetal sheep skin at 90 and 120 days of gestation, respectively. Note the resemblance of the epidermis in **a** and **b** with **g** fetal skin at 90 days of gestation. It contains the typical round cells with large and clear cytoplasm typically seen in early fetal skin. In contrary, **e** depicts an adult-type multilayered and stratified epidermis. **a**, **b**, **e** Substitutes produced with CnT-09 alone or in combination with supplemented DMEM yielded the best results

was used in the upper compartment after KC seeding and DMEM/10 % LS in the lower compartment, a stratified epidermis of eight to ten layers developed (Fig. 1e), strongly resembling tissue-engineered adult human dermo-epidermal skin substitutes. On fibrin-based gels, a very fragile monolayer, which usually ruptured during sample preparation, could be observed (Fig. 1f).

The compressed collagen-gels showed good handling properties and were felt to be stable enough to potentially survive open intrauterine exposure, while fibrin gels were too soft and fragile due to their predominantly aqueous content.

All collagen-based gels demonstrated a strong tendency to contract eight to ten days after gel production. The combination of collagen-based gels with Integra^{TM} dermal template reduced contraction, but rolling-in at the borders occurred nonetheless. On the contrary, contraction was not observed in fibrin-based gels. This was attributable to the strong adherence of the fibrin gel to the insert membrane. However, when the membrane was detached from the insert, contraction occurred all the same.

Immunohistochemistry

All epithelial markers and the marker for the basal lamina were expressed at 90 days as well as at 120 days GA (Figs. 2, 3). The staining pattern followed the well-known pattern usually found in human postnatal skin. K5 (Figs. 2a, 3a), K14 (Figs. 2c, 3c), and K15 (Figs. 2d, 3d) were expressed in the basal epidermis, K10 (Figs. 2b, 3b) in the suprabasal epidermis. At 90 days GA, the top layer of the epidermis stained positive for Loricrin (Fig. 2e). Whether this was specific periderm staining or unspecific sebum staining was not identifiable. At 120 days GA, the skin showed a distinct cornified layer with expression of Loricrin in the stratum corneum as well as in the upper intermediate layers (Fig. 3e). Laminin 1&2 stained the basal membrane of epidermis and of vessels and hair follicles within the dermis (Figs. 2f, 3f).

In the fDESS, K5 (Fig. 4a) and K14 (Fig. 4c) were strongly positive throughout the whole epidermis. K10 (Fig. 4b) was only expressed in the suprabasal layers whereas K15 was negative (Fig. 4d). Loricrin was detectable in the stratum corneum and in the uppermost intermediate layers (Fig. 4e). There was a weak but continuous staining for Laminin 1&2 (Fig. 4f), indicating deposition of a basal lamina-like structure.

Discussion

This study demonstrates that collagen-gel based, ovine fetal cell-derived skin analogues with near normal anatomy can be engineered in vitro. We have chosen to use ovine fetal cells in view of a future experimental in vivo autologous transplantation of such fDESS in the well-established ovine spina bifida model [4]. Although here, we have used fetal sheep cells, it is well conceivable that, envisioning application in humans, an analogous human fDESS can be engineered similarly, especially when considering that there is much more experience with human rather than sheep tissue engineering of skin [5].

When examining different cell culture media and gel types, collagen-based gels produced with CnT-09 yielded

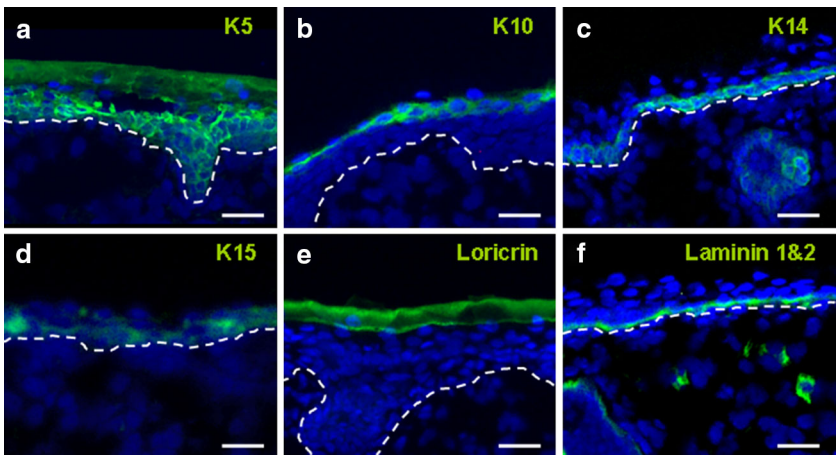

Fig. 2 Immunohistochemical staining of fetal sheep skin at 90 days of gestation. *Scale bar* 25 µm. The *dashed line* indicates the dermo-epidermal junction. Markers (all in *green*) of the epidermal compartment and of the basal membrane show the same pattern as seen in human epidermis. **a** K5 is expressed mainly basally, **b** K10 suprabasally. **c** K14 and **d** K15 stain positive in the stratum basale, although K15 is weak. **e** Positive Loricrin staining of the periderm in fetal skin of 90 days gestational age is to be expected, here it remains unclear whether this is a specific periderm staining or an unspecific sebum staining. **f** Laminin 1&2 is clearly positive in the basal membrane of the epidermis, vessels, and hair follicles

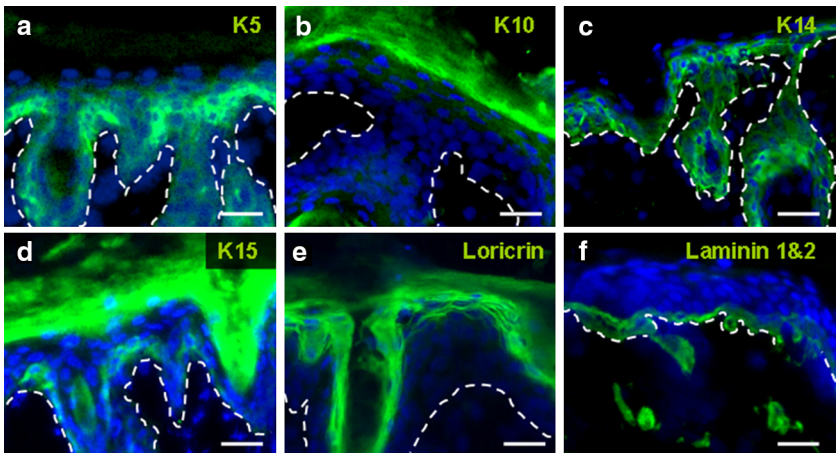

Fig. 3 Immunohistochemical staining of fetal sheep skin at 120 days of gestation. *Scale bar* 25 µm. The *dashed line* indicates the dermo-epidermal junction. The same staining pattern is seen as at 90 days of gestation (Fig. 2). **a** K5 is expressed mainly basally and **b** K10 suprabasally. **c** K14 stains positive in the stratum basale. **d** Specific staining of K15 is confined to the basal layer. The stratum corneum shows unspecific staining. **e** Clear positive Loricrin staining of the stratum corneum and the uppermost intermediate layers. **f** Expression of Laminin 1&2 in the basal membrane of epidermis, vessels and hair follicles

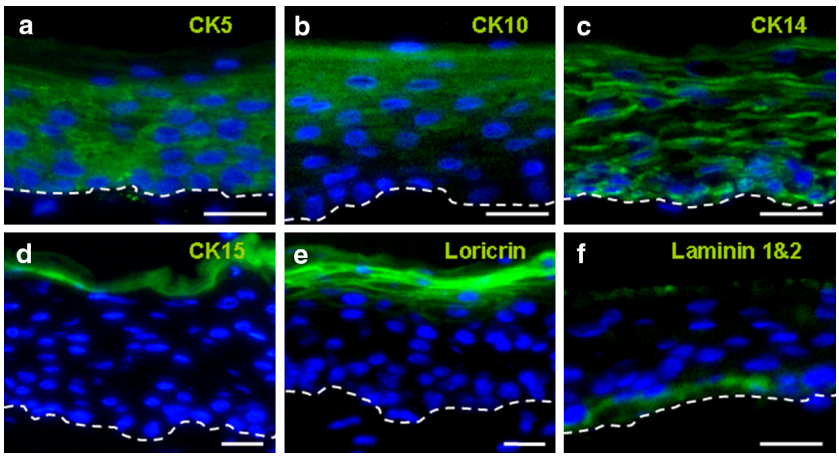

Fig. 4 Immunohistochemical staining of the dermo-epidermal skin substitute cultured with CnT-09/supplemented DMEM (H&E in Fig. 1e). The *dashed line* indicates the dermo-epidermal junction. All markers are depicted in *green*. **a** K5 and **c** K14 are strongly positive throughout the whole epidermis, indicating a "wound-like" situation with non-established homeostasis. **b** K10 is being expressed in the upper layers of the epidermis, indicating differentiation and stratification. **d** The stratum corneum has stained unspecifically; K15 is negative (as expected). **e** Loricrin is expressed in the granular and cornified layers, signaling differentiation and stratification. **f** Laminin 1&2 indicates that a basal membrane has been deposited

the best results in the 3D culture. According to the manufacturer, CnT-09 "allows efficient isolation and extended in vitro culture of canine and ovine keratinocytes". We noticed that in the 2D culture, CnT-09 induced differentiation of the fetal ovine KC. Therefore, CnT-57, a proliferation medium for human KC, was used for KC expansion prior to seeding. Interestingly, the fDESS obtained with the combination of CnT-09 and DMEM/10 % LS was clearly different from fetal skin morphology seen at the GA of the used cells (90 days) or at the expected chronological age (105 days = 90 days GA + 15 days substitute production). The epidermis differentiated to an adult-type epidermis consisting of several layers and a well-developed stratum corneum. The same was observed by Bickenbach and Holbrook [6] when culturing fetal skin in organ cultures. They noticed that fetal epidermis differentiated from three layers (basal, intermediate, and periderm) to an adult-type epidermis consisting of a basal layer, two to three spinous layers, one or two granular and several cornified layers.

We analyzed the expression patterns of proteins indicative for epidermal differentiation and homeostasis [7]. In fetal sheep skin, marker patterns were similar to those seen in human fetal and postnatal skin. In humans, K5 and K14 start to be expressed at 8 weeks GA, when the epidermis consists of basal layer and periderm [8, 9]. In the normal human homeostatic epidermis, K5 and K14 are expressed in the basal layer and the first suprabasal layers. In wound

edges, however, expression of K5 and K14 can be induced in migrating KC [10]. In our fDESS, K5 and K14 were strongly expressed suprabasally, indicating a "wound-like" situation with non-established homeostasis.

K10, a differentiation marker found suprabasally in postnatal human skin [10], is first induced between 9 and 10 weeks GA, when a third layer in between the stratum basale and the periderm, the intermediate layer, is formed [9]. We observed the same pattern in fetal sheep skin. In fDESS, K10 was also expressed suprabasally, hence, implying some degree of differentiation and stratification.

K15 is almost exclusively expressed in basal KC in a homeostatic human epidermis. It, therefore, serves as a dependable indicator for epidermal homeostasis [3]. It is absent during wound healing [11, 12] and is not expressed in dermo-epidermal skin equivalents before transplantation, but becomes upregulated in the stratum basale after transplantation [3, 7]. K15 was expressed in fetal skin at 90 and 120 days GA, but, as expected, not in fDESS.

Periderm cells are unique to embryonic and fetal skin and form the cornified cell envelope before keratinization occurs at 160 days GA [13]. Loricrin is one of the major precursor proteins of the cornified cell envelope and it is restricted to the periderm [13]. It is expressed from the time the three-layered epidermis is formed. After disappearance of the periderm and appearance of interfollicular keratinization, Loricrin is expressed in the granular and cornified layers [13]. In our study, Loricrin was expressed in fetal sheep skin and fDESS, again pointing out differentiation and stratification of the tissue-engineered epidermis. Clearly, the pattern seen in the fDESS was adultlike.

Laminin 1&2 stained positive in fDESS, demonstrating that a continuous basal lamina-like structure to anchor the epidermis was present. Taken together, there is convincing evidence from fDESS histology and immunohistochemistry that a mature, although not completely homeostatic, adultlike epidermis is formed in vitro. These findings are in contrast to the work of Haake and Cooklis [14], where under none of the conditions tested a mature epidermis was formed. In their study, the epidermal equivalent remained parakeratotic, and distribution patterns of K1 and K10 followed an aberrant organization with individual but dispersed stained cells in a disorganized epidermis. A possible explanation for these different findings may be the different culturing conditions and the different age of the fetal cells used.

While fDESS consistency using compressed collagen-based gels appeared ideal for surgical handling in utero, gel contraction caused considerable size reduction and increased thickness, thus representing a potentially severe drawback in view of an application for spina bifida repair. Interestingly, when using the same tissue engineering protocol with human postnatal foreskin FB, contraction is minimal. The positive effect of gel compression on stability and contraction in human collagen-gel-based skin substitutes has been previously published by our group [2]. It can, therefore, be deduced that gel contraction in this study is caused by the ovine fetal cells, particularly FB, as contraction occurred also in control gels where KC seeding was omitted. Haake and Cooklis [14] observed that fetal FB contracted a dermal equivalent composed of rat tail type I collagen to a greater extent than neonatal FB (unpublished observation). But they also noticed contraction in fetal epidermis cultured on a neonatal dermal equivalent. As explanation, they suggested a combination of factors in the epidermis such as high level of fetal KC migration over the dermal equivalent, localized proliferation, and apoptosis. In our series, contraction of the epidermis on the dermal equivalent was not seen. The epidermis always covered the whole gel.

The combination of collagen-based gels with Integra™ dermal template proved to reduce contraction. This can be explicated by the fact that the dermal template itself cannot loose volume and the collagen gel itself cannot retract from the pores of the template it has solidified in. The contraction forces, however, led to a rolling-in of the borders.

The envisioned clinical application of tissue-engineered autologous fetal skin is in utero repair of large spina bifida lesions in human fetuses where primary skin closure is not possible. Up to now, acellular dermal matrices such as Integra™ (Integra LifeScience, Plainsboro, NJ, USA) or AlloDerm® (LifeCell™, Bridgewater, NJ, USA) have been clinically used for skin defect coverage [15, 16]. Although these non-viable scaffolds usually provide sufficient closure for the remainder of pregnancy, they are not optimal. In case of Integra™, the silicon top layer guarantees watertight coverage for at least several weeks before it is detached from the underlying and now well-vascularized neodermis that has gradually formed. But the silicon foil apparently also hinders KC migration and thus the formation of an epidermis. Hence, these patients require soon after birth at least a split thickness skin transplantation over the Integra™-derived neodermis [15]. There is no specific literature on outcomes after using AlloDerm®. This product has been used for skin closure in fetal spina bifida repair in large series and has not compromised fetal repair (MOMS trial), but complete epithelialization is not always present at birth and additional wound management may be necessary. In the best case scenario, fDESS would offer the possibility of a single step, autologous, and thus definitive in utero skin reconstruction. Obviously, a minute skin biopsy would have to be harvested about three weeks earlier, ideally when the obligatory diagnostic amniocentesis is performed.

Hypothetically, fDESS could, under particular circumstances, also be used in the neonatal period, e.g., for

postnatal repair of large spina bifida lesions (fDESS cultured for prenatal repair that was finally not performed) or, alternatively, as proposed by Fauza et al. [17], for other indications such as large body wall defects. Last, gene-modified tissue-engineered fetal skin could be made available for newborns with genetic skin diseases, after prenatal skin biopsy has confirmed the diagnosis and was then used as cell source for culturing. Of note, fetal skin biopsies are performed since many years for prenatal diagnosis of genetic skin diseases [18].

In conclusion, this study provides evidence that collagen-gel-based ovine fetal cell-derived skin analogues with near normal cutaneous anatomy can be engineered in vitro. This compelling progress raises the possibility of using laboratory grown fetal skin during fetal surgery for spina bifida to achieve autologous, instant, single step, and definitive skin reconstruction in situations where primary autochthonous skin closure is not possible.

Acknowledgments We are particularly grateful to the Gottfried and Julia Bangerter-Rhyner Foundation, to the Children's Research Center of the University Children's Hospital Zurich and to a private sponsor that prefers to remain anonymous for their generous financial support and interest in our work.

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

References

1. Adzick NS, Thom EA, Spong CY et al (2011) A randomized trial of prenatal versus postnatal repair of myelomeningocele. N Engl J Med 364:993–1004
2. Braziulis E, Diezi M, Biedermann T et al (2011) Modified plastic compression of collagen hydrogels provides an ideal matrix for clinically applicable skin substitutes. Tissue Eng Part C Methods 18(6):464–474
3. Pontiggia L, Biedermann T, Meuli M et al (2009) Markers to evaluate the quality and self-renewing potential of engineered human skin substitutes in vitro and after transplantation. J Invest Dermatol 129:480–490
4. Meuli M, Meuli-Simmen C, Yingling CD et al (1995) Creation of myelomeningocele in utero: a model of functional damage from spinal cord exposure in fetal sheep. J Pediatr Surg 30:1028–1032 (discussion 1032–1023)
5. Marino D, Reichmann E, Meuli M (2014) Skingineering. Eur J Pediatr Surg 24:205–213
6. Bickenbach JR, Holbrook KA (1986) Proliferation of human embryonic and fetal epidermal cells in organ culture. Am J Anat 177:97–106
7. Biedermann T, Pontiggia L, Böttcher-Haberzeth S et al (2010) Human eccrine sweat gland cells can reconstitute a stratified epidermis. J Invest Dermatol 130:1996–2009
8. Dale BA, Holbrook KA, Kimball JR et al (1985) Expression of epidermal keratins and filaggrin during human fetal skin development. J Cell Biol 101:1257–1269
9. Coolen NA, Schouten KC, Middelkoop E et al (2010) Comparison between human fetal and adult skin. Arch Dermatol Res 302:47–55
10. Patel GK, Wilson CH, Harding KG et al (2006) Numerous keratinocyte subtypes involved in wound re-epithelialization. J Invest Dermatol 126:497–502
11. Waseem A, Dogan B, Tidman N et al (1999) Keratin 15 expression in stratified epithelia: downregulation in activated keratinocytes. J Invest Dermatol 112:362–369
12. Porter RM, Lunny DP, Ogden PH et al (2000) K15 expression implies lateral differentiation within stratified epithelial basal cells. Lab Invest 80:1701–1710
13. Akiyama M, Smith LT, Yoneda K et al (1999) Periderm cells form cornified cell envelope in their regression process during human epidermal development. J Invest Dermatol 112:903–909
14. Haake AR, Cooklis M (1997) Incomplete differentiation of fetal keratinocytes in the skin equivalent leads to the default pathway of apoptosis. Exp Cell Res 231:83–95
15. Meuli M, Meuli-Simmen C, Flake AW et al (2013) Premiere use of Integra artificial skin to close an extensive fetal skin defect during open in utero repair of myelomeningocele. Pediatr Surg Int 29:1321–1326
16. Mazzola CA, Albright AL, Sutton LN et al (2002) Dermoid inclusion cysts and early spinal cord tethering after fetal surgery for myelomeningocele. N Engl J Med 347:256–259
17. Fauza DO, Fishman SJ, Mehegan K et al (1998) Videofetoscopically assisted fetal tissue engineering: skin replacement. J Pediatr Surg 33:357–361
18. Holbrook KA, Smith LT, Elias S (1993) Prenatal diagnosis of genetic skin disease using fetal skin biopsy samples. Arch Dermatol 129:1437–1454