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REVIEW

Skin and diabetes mellitus: what do we know?

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Abstract Diabetes mellitus (DM) is becoming increasingly prevalent worldwide. Although major complications of this condition involve kidney, retina and peripheral nerves, the skin of diabetic patients is also frequently injured. Hence, interest is mounting in the definition of the structural and molecular profile of non-complicated diabetic skin, i.e., before injuries occur. Most of the available knowledge in this area has been obtained relatively recently and, in part, derives from various diabetic animal models. These include both insulin-dependent and insulin-resistant models. Structural work in human diabetic skin has also been carried out by means of tissue samples or of non-invasive methods. Indications have indeed been found for molecular/structural changes in diabetic skin. However, the overall picture that emerges is heterogeneous, incomplete and often contradictory and many questions remain unanswered. This review aims to detail, as much as possible, the various pieces of current knowledge in a systematic and synoptic manner. This should aid the identification of areas in which key questions are still open and more research is needed. A comprehensive understanding of this field could help in determining molecular targets for the prevention and treatment of skin injuries in DM and markers for the monitoring of cutaneous and systemic aspects of the disease. Additionally, with the increasing development of non-invasive optics-based deep-tissue-imaging diagnostic technologies, precise knowledge of cutaneous texture and molecular structure becomes an important pre-requisite for the use of such methods in diabetic patients.

Keywords Non-complicated skin · Diabetes mellitus · Cutaneous structure · Molecular composition · Diabetic skin

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Abbreviations

AGEs	Advanced glycation endproducts
AX	Alloxan
BB rats	Bio-breeding rats
BM	Basement membrane
cm-OCT	Correlation mapping optical coherence tomography
CYP	Cytochrome P450
DM	Diabetes mellitus
DTA	Diphteria toxin A-chain
ECM	Extracellular matrix
GFOGER	Glycine—phenylanaline—hydrohyproline—glycine—glutamic acid—arginine
GLUT4	Glucose transporter 4
HFD	High-fat diet
MMPs	Matrix metalloproteinases
NOD mice	Non-obese-diabetic mice
OCT	Optical coherence tomography
OLETF rats	Otsuka Long Evans Tokushima fatty rats
RGD	Arginine—glycine—aspartic acid
SAF	Skin autofluorescence
STZ	Streptozotocin
TEWL	Trans-epidermal water loss
TIMP	Tissue inhibitor of matrix metalloproteinases
TSNO mice	Tsumura-Suzuki non-obesity mice
TSOD mice	Tsumura-Suzuki obese diabetic mice
UCP1	Uncoupling protein 1

General aspects of diabetes mellitus

Diabetes mellitus (DM) is a chronic and systemic condition characterised by hyperglycaemia and severe complications such as retinopathy, nephropathy and neuropathy (American

Diabetes Association 2013). Clinically, the most common forms are type 1 DM (insulin-dependent) and type 2 DM (non-insulin-dependent, ~90 % DM), the latter of which is associated with obesity and insulin resistance (Zimmet et al. 2001; Lam and LeRoith 2012). Prevalence of DM is constantly increasing. In 2011, the total worldwide population affected by the disease was reported to be in excess of 350,000,000 and this figure will increase to around half a billion by 2030 (Lam and LeRoith 2012). Such an order of magnitude was predicted at the beginning of the 2000s, when DM was defined as "one of the main threats to human health of the 21st century" (Zimmet et al. 2001). Accordingly, DM has a high socio-economic impact. For example, the financial burden of a patient over the age of 65 with type 2 DM has been estimated to be nearly double that of patients in the same age group without type 2 DM (O'Shea et al. 2013).

Skin involvement in DM

The skin (Fig. 1) is variably but often, affected during DM. Reported figures indicate that about 1/3 to nearly all diabetic patients experience cutaneous complications (Aye and Masson 2002; Wohlrab et al. 2007; Bristow 2008; van Hattem et al. 2008). The most severe cutaneous lesions are chronic ulcers that form as a consequence of the poor healing potential of diabetic skin (Falanga 2005). These can frequently become infected, thus leading to amputation (Ngo et al. 2005; Eaglstein and Callen 2009). In the Republic of Ireland in 2009, for example, the total number of lower limb amputations specifically related to DM was 175.7 per 100,000 diabetic patients, compared with lower limb amputations not related to DM, which had a frequency of 9.2 per 100,000 non-diabetic patients (Buckley et al. 2012).

A large amount of work on skin in DM has primarily been focused on the management of the diabetic foot and on experimental wound healing (e.g., Greenhalgh et al. 1990; Aye and Masson 2002; Dinh and Veves 2005; Rodgers et al. 2006; Schramm et al. 2006; Berdal et al. 2011; Bermudez et al. 2011; Zhao et al. 2012). Conversely, non-injured skin in DM is relatively understudied. As a consequence, knowledge of the in vivo structure and molecular composition of non-injured diabetic skin is fragmentary, most being relatively recent and to a wide extent derived from various diabetic animal models. This body of work provides an indication for molecular/structural cutaneous changes in DM. However, actual molecular/structural features of diabetic skin are currently poorly defined and a number of questions remain unanswered.

This review will focus on the structural and molecular knowledge of non-injured diabetic skin. It attempts to gather various pieces of current data and to collate them systematically into a synoptic view (Tables 1, 2). This will help to identify areas in which key questions are still open and more research is needed. Other aspects, such as clinical conditions of dermatologic interest associated with DM and diabetic wound healing will only be referred to in order to place the actual content of the review into its appropriate context. For a more comprehensive overview of these aspects, the reader can refer to a broad body of existing literature (e.g., Greenhalgh et al. 1990; Lipsky et al. 2000; Aye and Masson 2002; Dinh and Veves 2005; Rodgers et al. 2006; Schramm et al. 2006; Wohlrab et al. 2007; Bristow 2008; Larsen et al. 2008; van Hattem et al. 2008; Berdal et al. 2011; Bermudez et al. 2011; Ragunatha et al. 2011; Zhao et al. 2012; Behm et al. 2012). The review begins with a brief introduction to the diabetic animal models used for skin analysis. The outcome of the individual skin studies will then be discussed in the subsequent sections, in the context of the main topic, starting from the epidermal barrier and systematically moving (anatomically speaking) deeper in the direction of the dermis and dermal microvessels.

Animal models used in the study of diabetic skin

Since the end of the nineteenth century, when von Mering and Minkowski carried out the first radical pancreatectomy on dogs (von Mering and Minkowski 1890), a series of animal models for the study of DM have been developed (Rees and Alcolado 2005; Chatzizgeorgiou et al. 2009; King 2012). In this section, models that have been used in investigations into non-injured skin in DM will be briefly introduced. For more details and for a wider view on the use of animal models in DM, the reader can refer to several excellent review papers (e.g., Rees and Alcolado 2005; Chatzizgeorgiou et al. 2009; King 2012).

Type 1 diabetes models. The generation of insulin-dependent DM in experimental animals for skin studies has typically been carried out by means of streptozotocin (STZ) or alloxan (AX) treatment.

STZ treatment This drug leads to β-cell destruction and, thus, the animals develop insulopaenia and hyperglycaemia (Chatzizgeorgiou et al. 2009; King 2012). However, STZ treatment can also be followed by islet regeneration, with obvious consequences on glycaemia and this must be taken into account when using this model (King 2012). In studies of non-injured skin, STZ treatment is the most frequently used among the insulin-dependent models and has been employed in rats (Craig et al. 1998; Kiliçaslan and Özer 2009; Chen et al. 2010; Takahashi and Takasu 2011; Tellechea et al. 2013), mice (Sakai et al. 2003; Tellechea et al. 2013) and baboons (Heffernan et al. 1996).

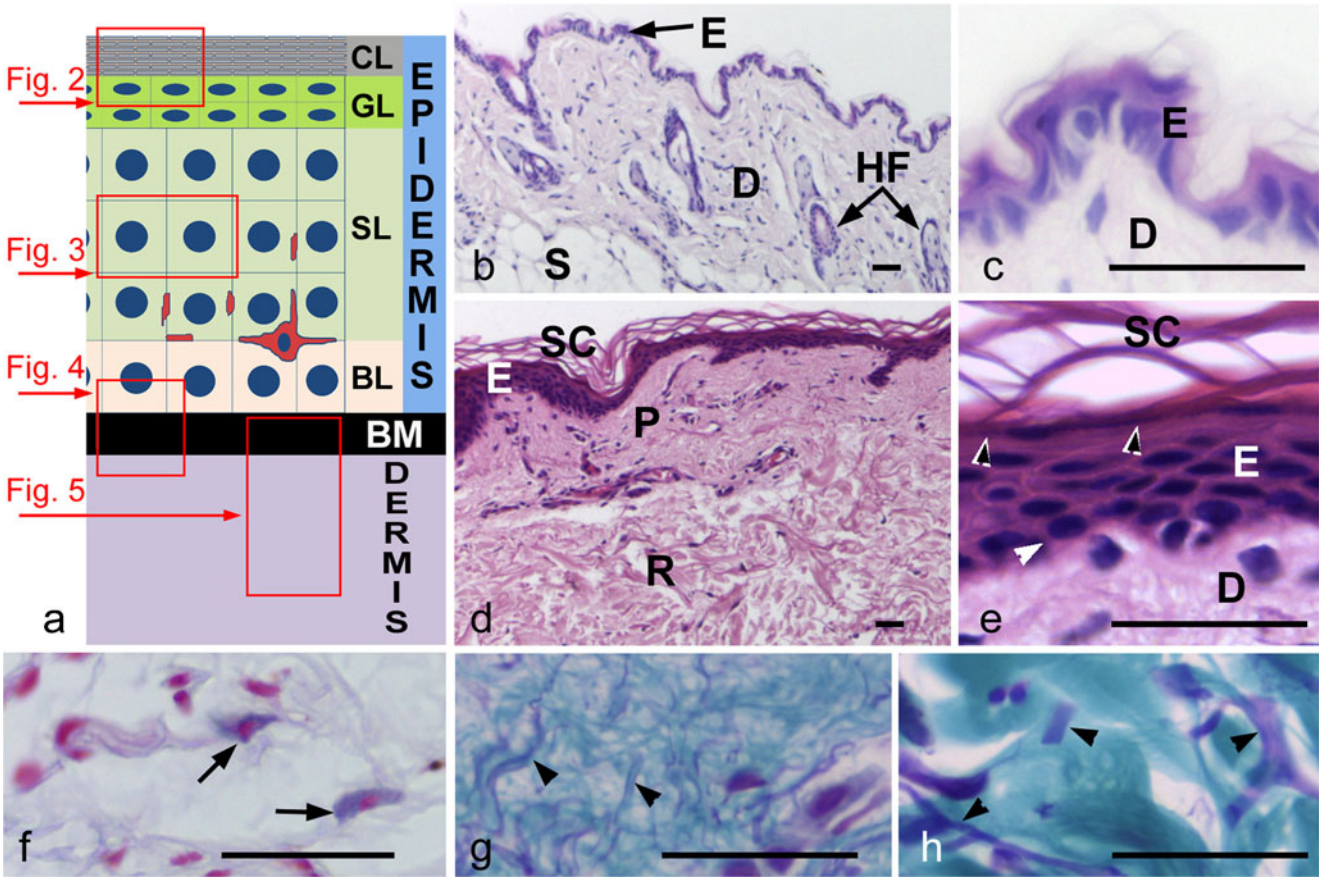

Fig. 1 Skin overview (**a**) showing a representation of general cutaneous organisation according to Simpson et al. (2011). The skin is composed of the epidermis and dermis separated (and united) by the epidermal basement membrane (*BM*). The epidermis is mainly composed of keratinocytes that organise themselves to form four layers, namely (from the BM to the skin surface), the basal layer or stratum basale (*BL*), spinous layer or stratum spinosum (*SL*), granular layer or stratum granulosum (*GL*) and cornified layer or stratum corneum (*CL* or SC). This representation gives a general orientation for Figs. 2, 3, 4 and 5 in which the skin structure is presented in more detail in the individual regions corresponding to those in the *red frames*. Intercalated with keratinocytes, other cell types are present in the epidermal layer, e.g., dendiritc cells (*red cell*). These cells extend their processes between the keratinocytes and play an important role in host defence. For simplicity, other cell types usually found in the epidermis (e.g., melanocytes) are omitted; only dendritic cells are depicted here, as they will be discussed with regard to the barrier function of skin. **b–e** Skin samples stained by haematoxylin-eosin (*HF* hair follicles with associated sebaceous glands, *E* epidermis, *D* dermis, *S* subcutis, *SC* stratum corneum). **b**, **c** Murine back skin. **d**, **e** Human skin, mammary region. Note that, although the general structure of the epidermis also applies to murine skin, the layers are much less distinct in the murine epidermis than in human skin at the light microscope level (cf. **c**, **e**). In human skin at the light microscope level, a basal layer can be recognised (**e**, *white arrowhead*) composed of cuboidal cells. Towards the skin surface (just underneath the cornified layer), a granular layer (**e**, *black/white arrowheads*) can be seen in which the cells are flat with more intense staining in their cytoplasm attributable to fine granula. Between the two layers, the spinous layer (intermediate layer) has cells that are quadrangular/polygonal that tend to become flat towards the skin surface. In the dermis of human skin, two distinct layers can be observed at the light microscope level: a more superficial layer, namely the papillary layer (*P*) and a deeper layer called the reticular layer (*R*). The papillary layer contains a much finer network of fibrillar extracellular matrix components, which become coarser and denser in the reticular layer. Although also in murine skin the fibres tend to become larger and more densely packed in the deep dermis (as seen ultrastructurally), such a distinction into two layers is not obvious at the light microscope level (cf. **b**, **d**). **f** Two mast cells stained by alcian blue (*arrows*) between a number of other cells (mostly fibroblasts, see *red* cell nuclei) in murine dermis. **g**, **h** Higher power views of papillary and reticular dermis, respectively, of human skin (same sample as in **d**, **e**) stained by the trichromic method to highlight the fibrous components of the connective tissue, namely collagen (*green*) and elastic fibres (*dark blue/grey*, *arrowheads*). Note the difference in size of the fibrous components and the organisation of the overall network. *Bars* 30 μm

AX treatment This drug is also delivered to β-cells where it triggers their destruction (Rees and Alcolado 2005; Chatzizgeorgiou et al. 2009; King 2012). In studies on non-injured skin, AX treatment has been used in rabbits (Tellechea et al. 2013) and in mice (Sakai et al. 2003; Ye et al. 2013). However, in the paper of Sakai et al. (2003) in which both AX and STZ models have been investigated, the results on skin structure do not consistently refer to the AX model, whereas they are consistently described in relation to the STZ model. This is probably because DM induction is more complicated following AX treatment as a result of its higher toxicity (King 2012; Sakai et al. 2003).

In general, despite being suitable for generating insulin-dependent diabetic animals, neither model has a strong

Table 1 Summary of the literature on the most relevant epidermal features in diabetes mellitus (DM). The age of db/db mice is reported here as it is of relevance for the development of DM and might become relevant in the interpretation and comparison of the results of the studies that use this mouse model (*OLETF rats* Otsuka Long Evans Tokushima fatty rats, *STZ* streptozotocin, *HFD* high-fat diet)

Character	Effect in DM	Reference	Human/model (method)
Hydration state	Decreased	Sakai et al. 2005	Patients
		Park et al. 2011	OLETF rats
	No alteration	Seirafi et al. 2009	Patients
Trans-epidermal water loss	Not altered	Sakai et al. 2005	Patients
		Seirafi et al. 2009	Patients
		Sakai et al. 2003	STZ mice
		Park et al. 2011	OLETF rats
Filaggrin	Alteration	Thyssen et al. 2011	Patients (genetic defects)
	Normal expression	Sakai et al. 2003	STZ mice (Western blot)
		Park et al. 2011	OLEFT rats (immunohistochemistry)
Other possible signs of barrier defect	Increased inflammatory infiltration	Tellechea et al. 2013	Patients and various type 1 animal models
	Reduced functionality of gamma-delta T cells	Taylor et al. 2011	12-week-old db/db and HFD mice
	Reduced antimicrobial peptides, lamellar bodies, stratum corneum lipids and corresponding enzymes	Park et al. 2011	OLETF rats
Epidermal differentiation	No alteration in markers	Sakai et al. 2003	STZ mice (keratin 1, 5, 10, loricrin; Western blot)
		Park et al. 2011	OLETF rats (involucrin, loricrin; immunohistochemistry)
		Park et al. 2011	OLETF rats (involucrin, loricrin; immunohistochemistry)
	Alterations in differentiation markers	Taylor et al. 2011	12-week-old db/db & HFD mice (keratin 1, 5, E-cadherin; immunohistochemistry/Western blot)
		Rodgers et al. 2006	4– to 6-week-old db/db mice (keratin 1, 2 and keratin-associated proteins; gene array)
Keratinocyte proliferation	No alteration	Park et al. 2011	OLETF rats
		Taylor et al. 2011	6-week-old db/db mice
	Reduced proliferation	Sakai et al. 2003	STZ mice
		Chen et al. 2010	Human keratinocytes in vitro exposed to glycation modified albumin
		Taylor et al. 2011	12-week-old db/db and HFD mice
Epidermal thickness	No alteration	Park et al. 2011	OLETF rats
		Taylor et al. 2011	6-week-old db/db mice
		Zakharov et al. 2010	Patients (non-invasive optical coherence)
	Thinner epidermis	Sakai et al. 2003	STZ mice
		Chen et al. 2010	STZ rats
		Taylor et al. 2011	12-week-old db/db and HFD mice
	Thicker epidermis	Bertheim et al. 2002	Patients with associated marked depletion of hyaluronic acid

autoimmune component (Chatzizgeorgiou et al. 2009), which otherwise characterises the vast majority of human type 1 DM (American Diabetes Association 2013). Nevertheless, in several settings that do not particularly focus on the immune aspects of the disease, both STZ and AX models are widely used as the model of choice. Models with spontaneous autoimmune destruction of β-cells exist, e.g., the non-obese-diabetic (NOD) mouse and the bio-breeding (BB) rat. However, although these models bear the autoimmune component that is an important aspect of the human disease, they also

Table 2 Summary of the most relevant dermal aspects of diabetic skin. The age of db/db mice is reported as it is of relevance for the development of the diabetes and also might become relevant in the interpretation and comparison of the results of the studies that use this mouse model (*ECM* extracellular matrix, *TSOD mice* Tsumura-Suzuki obese diabetic mice, *STZ* streptozotocin, *MMP* matrix metalloproteinase, *TIMP* tissue inhibitor of matrix metalloproteinases, *AX* alloxan, *UCP1* uncoupling protein 1, *DTA* diphtheria toxin A)

Character	Effect on diabetic skin	Reference	Human/model/article type (method)
Skin mechanical properties	Diabetic skin is mechanically inferior	Liao et al. 2009	Artificially glycated matrix
		Bermudez et al. 2011	Skin samples of patients and of 10– to 14-week-old db/db mice
		Ibuki et al. 2012	TSOD mice
Dermal collagen organisation	No alteration	Heffernan et al. 1996	STZ baboon (X-ray diffraction)
		Tahrani et al. 2012	Diabetic patients with no complications (histology, light microscopy)
	Disorganised dermal collagen deposition	Tahrani et al. 2012	Diabetic patients with complications (histology, light microscopy)
		Ibuki et al. 2012	TSOD mice (scanning electron microscopy)
Collagen type I	Reduced production/deposition	Craig et al. 1998	STZ rats
		Ye et al. 2013	AX mice
		Bermudez et al. 2011	10– to 14-week-old db/db mice
		Tahrani et al. 2012	Patients; worse depletion in presence of complications
	Reduced gene expression	Rodgers et al. 2006	4– to 6-week-old db/db mice
	Higher gene expression	Bermudez et al. 2011	10– to 14-week-old db/db mice
Collagen types III and V	Reduced gene expression	Rodgers et al. 2006	4– to 6-week-old db/db mice
	Higher gene expression	Bermudez et al. 2011	10– to 14-week-old db/db mice
MMP9	Increase after stimulation by age in vitro	Fujimoto et al. 2010	Commercially available human keratinocytes
		Zhu et al. 2011	HaCat cells
	Increased activity	Takahashi and Takasu 2011	STZ rats
	Decreased gene expression and no activity	Bermudez et al. 2011	10– to 14-week-old db/db mice
	Decreased protein level	Bermudez et al. 2011	10– to 14-week-old db/db mice and patients
	No change in gene expression	Rodgers et al. 2006	4– to 6-week-old db/db mice
		Ibuki et al. 2012	TSOD mice, subcutis
	No change in quantity but more active	Tahrani et al. 2012	Patients
General proteolytic balance for the dermal ECM	Higher	Tahrani et al. 2012	Patients, activation of MMP 1, 2, 9 (see also above), reduced levels of TIMP1
		Takahashi and Takasu 2011	STZ rats, higher activity of MMP2, 9 and of hyaluronidase
		Ye et al. 2013	AX mice, increased expression of MMP13 and decreased expression of TIMP1
		Ibuki et al. 2012	TSOD mice, subcutis; due to increased expression of MMP2, 14
	Lower	Bermudez et al. 2011	10– to 14-week-old db/db mice, depletion of MMP9 (see above) and increased TIMP1
	No change	Rodgers et al. 2006	4– to 6-week-old db/db mice, no change in expression of relevant genes (array)
Microvascular density	Increase	Tellechea et al. 2013	Patients and various type 1 animal models (immunohistochemistry)
	Decrease	Algenstaedt et al. 2003	UCP1/DTA mice (in vivo microscopy)
		Schaefer et al. 2010	UCP1/DTA mice (in vivo microscopy)

Table 2 (continued)

Character	Effect on diabetic skin	Reference	Human/model/article type (method)
		Ngo et al. 2005	Review
	No change	Aellen et al. 2012	Patients (in vivo capillaroscopy)
		Schramm et al. 2006	Review
Microvascular wall	Thickening	Banson and Lacy 1964; Moore and Frew 1965; Braverman and Keh-Yen 1984; Braverman et al. 1990	Patients
		Dinh and Veves 2005; Ngo et al. 2005; Schramm et al. 2006	Reviews
		Kiliçaslan and Özer 2009; Chen et al. 2010	STZ rats

have significant limitations, such as high maintenance costs (both models), low predictability of the development of diabetes in NOD mice or severe leucopaenia in BB rats (Chatzizgeorgiou et al. 2009; King 2012).

Type 2 diabetes models. The following section will briefly describe the insulin-resistant diabetic models that have been used to study the structure and molecular composition of non-injured skin in DM.

The mutant db/db mouse The db/db mouse has a spontaneous mutation in the leptin receptor gene. This mutation, when homozygous, on the genetic background C57BLKS/J, causes a phenotype that resembles many aspects of insulin-resistant DM in humans. Mice gradually lose sensitivity to insulin (around 2 weeks of age) and become hyperphagic, with obesity becoming evident at about 4 weeks of age. At 4–8 weeks of age, homozygous mutants develop hyperglycaemia. Other features include hyperinsulinaemia, polyuria, polydipsia, neuropathic and cardiovascular complications and poor wound healing. At about 20 weeks of age, health deterioration is severe and total life expectancy is about 8–10 months (http://jaxmice.jax.org/strain/000642.html; Greenhalgh et al. 1990; Kobayashi et al. 2000; Chatzizgeorgiou et al. 2009; Taylor et al. 2011; King 2012). This model has been extensively used in studies of experimental diabetic wound healing for many years (Greenhalgh et al. 1990; Rodgers et al. 2006; Berdal et al. 2011; Bermudez et al. 2011; Zhao et al. 2012). In structural studies of non-injured skin, this mouse model has been examined in three studies (Rodgers et al. 2006; Bermudez et al. 2011, and Taylor et al. 2011).

High-fat diet The high-fat diet (HFD) model generally involves placing C57BL/6 mice on a diet in which approximately 58 % of the caloric intake is derived from fat (King 2012). This leads to weight gain, insulin resistance and ultimately glucose intolerance (King 2012). A similar approach has been adopted in the only investigation performed on skin samples from obese HFD mice (Taylor et al. 2011).

Otsuka Long Evans Tokushima fatty rats Otsuka Long Evans Tokushima fatty (OLETF) rats were generated by selective breeding at the Tokushima Research Institute in Japan. In this model, male rats are more likely to develop DM than females (Rees and Alcolado 2005; Chatzizgeorgiou et al. 2009; King 2012). Susceptibility loci have been located on chromosomes 1, 7, 14 and X (Rees and Alcolado 2005) possibly explaining why males are more prone to the disease than females. Insulin-resistant DM starts to develop at around 4–5 months of age and is characterised by polyphagia, hypercholesterolemia, high blood triglycerides and hyperinsulinemia, all of which are accompanied by mild obesity (Rees and Alcolado 2005; Chatzizgeorgiou et al. 2009; King 2012). The initial phase of infiltration and destruction of pancreatic islets is followed by a hyperplastic phase, which after about 40 weeks of age evolves into a fibrotic stage (King 2012). Renal malfunction has also been reported for this rat strain (King 2012). With regard to non-injured skin analysis, this rat strain has been used in one study (Park et al. 2011).

Tsumura-Suzuki obese diabetic mice The Tsumura-Suzuki obese diabetic (TSOD) mouse (Suzuki et al. 1999) is a spontaneously obese in-bred mouse strain in which male mice also develop insulin-resistant DM (Hirayama et al. 1999; Suzuki et al. 1999). When this strain was generated, progeny from the same ancestors were obtained that did not develop obesity and diabetes. This strain was named the Tsumura-Suzuki non-obesity (TSNO) mouse and represents the control for studies on TSOD mice (Hirayama et al. 1999; Suzuki et al. 1999; Ibuki et al. 2012). At 4 weeks of age, male TSOD mice develop a progressive and significant increase in body weight and in glucose intolerance followed by hyperinsulinaemia (Hirayama et al. 1999). Several other phenotypic aspects of TSOD mice have been reported. At 12 weeks of age, TSOD

mice have a significantly higher body weight, subcutaneous fat level and fasting glucose than TSNO mice (Ibuki et al. 2012). High non-fasting glucose levels have been reported at 13 weeks of age and progressively increasing levels have been measured at 16 and 24 weeks (Hirayama et al. 1999). Iizuka et al. (2005) performed extensive analysis at 6, 12 and 18 months of age and reported several features of DM in TSOD compared with TSNO mice, including nephropatic and neuropathic complications (Iizuka et al. 2005). The reason why TSOD become diabetic whereas the other in-bred strain (TSNO) does not is not fully understood. However, TSOD mice have altered gene expression of hypothalamic neuropeptides and a reduction in nucleobindin 2 protein levels compared with TSNO mice (Miyata et al. 2012) and altered hepatic CYP expression (Kudo et al. 2009). Additionally, in response to insulin stimulation, TSOD mice have a reduced ability to translocate GLUT4 to the membrane in skeletal muscle and adipose cells (Miura et al. 2001). Finally, a genetic analysis performed on TSOD mice has revealed the presence of three major loci associated with DM on chromosomes 1, 2 and 11 (Hirayama et al. 1999). DM in TSOD mice does not progress to high levels of severity and these mice retain normal life expectancy and fertility (Hirayama et al. 1999). One structural study on non-injured skin has examined this model (Ibuki et al. 2012).

UCP1/DTA mice These mice were generated by the selective expression of the diphtheria toxin A (DTA) chain in brown adipose tissue under the control of the uncoupling protein 1 (UCP1) promoter. This results in brown adipose tissue depletion and obesity (Lowell et al. 1993). During the second postnatal month, mice develop hyperphagia, followed by obesity and high levels of cholesterol, triglycerides, glucose and insulin, although they retain fertility (Lowell et al. 1993). UCP-DTA mice have been used in two studies related to skin vasculature in DM (Algenstaedt et al. 2003; Schaefer et al. 2010).

The epidermal barrier in diabetic skin

An ongoing discussion relates to whether diabetic patients are more susceptible to infections and/or to a worse outcome of such diseases. Indeed, comorbidity might contribute to the higher infection risk in DM, even though its impact is difficult to assess (Jackson 2005; Knapp 2012). However, a number of large studies conducted in the United States, Canada, Australia and The Netherlands suggests that diabetic patients do indeed have a higher risk of infectious diseases, higher mortality and higher frequency of complications and hospitalisation attributable to infections (Geerlings et al. 2000; Shah and Hux 2003; Muller et al. 2005; Hamilton et al. 2013; Suaya et al. 2013). Moreover, success in treating *Staphylococcus aureus* infections by vancomicin and linezolid is less likely in diabetic patients compared with non-diabetic subjects (Lipsky et al. 2011). Interestingly, the level of *Staphylococcus aureus* has been reported to increase in non-lesional skin of the plantar surface of the feet of diabetic patients when compared with non-diabetic individuals (Redel et al. 2013) and that its cutaneous tropism is facilitated by an epidermal barrier defect (Wanke et al. 2013). Therefore, the question arises as to whether DM is associated with damage to the epidermal barrier (Fig. 2).

The diabetic condition does not seem to increase transepidermal water loss (TEWL) in patients (Koivukangas et al. 1999; Sakai et al. 2005; Seirafi et al. 2009). In particular, Sakai et al. (2005) reported that a decreased state of skin hydration is associated with high fasting plasma glucose but not with an increase in TEWL; they concluded that dehydration is associated with the state of hyperglycaemia but not with a defect in the epidermal barrier (Sakai et al. 2005). Interestingly, the same authors also reported a decrease in TEWL in association with higher levels of glycated haemoglobin and concluded that this is an effect of ageing unrelated to DM (Sakai et al. 2005). The authors also correlated lipid alterations in the stratum corneum with ageing and suggested that hyperglycaemia accelerates dehydration, which would otherwise occur in ageing and that sebum alterations probably contribute to this effect (Sakai et al. 2005). Interestingly, a more recent study reported that, whereas a decrease in sebum levels occurs on the forehead of diabetic patients, no alterations occur in the hydration status of the skin of diabetics (Seirafi et al. 2009). Possibly, climatic circumstances and the ethnic backgrounds of the patients examined in the two studies account for these discrepancies. However, given the lack of increase in TEWL in non-injured skin of diabetic patients, the conclusion was drawn, in both studies, that no barrier defect occurs in DM (Sakai et al. 2005; Seirafi et al. 2009).

Interestingly, in human skin, an association between genetic defects in filaggrin (a component of the epidermal barrier; McAleer and Irvine 2013) and diabetes has been made among the Danish population (Thyssen et al. 2011). This suggests a possible barrier defect in humans and contradicts the above studies. In support of this notion, a recent study that analysed, for the first time, the level of inflammation in diabetic skin, reported increased cellular infiltration in samples of human skin (both type 1 and 2 DM) and in samples of various animal models of insulin-dependent DM (Tellechea et al. 2013). This obviously suggests that a more permissive barrier in DM exists, rather than an unaltered one. However, no structural studies have ever been performed in human diabetic skin to address the issue of the barrier specifically. In contrast, some structural work has been carried out in rodents (STZ mouse, Sakai et al. 2003; OLETF rat, Park et al. 2011). Both these studies reported normal filaggrin expression and higher dehydration of the stratum corneum of the diabetic animals without a significant

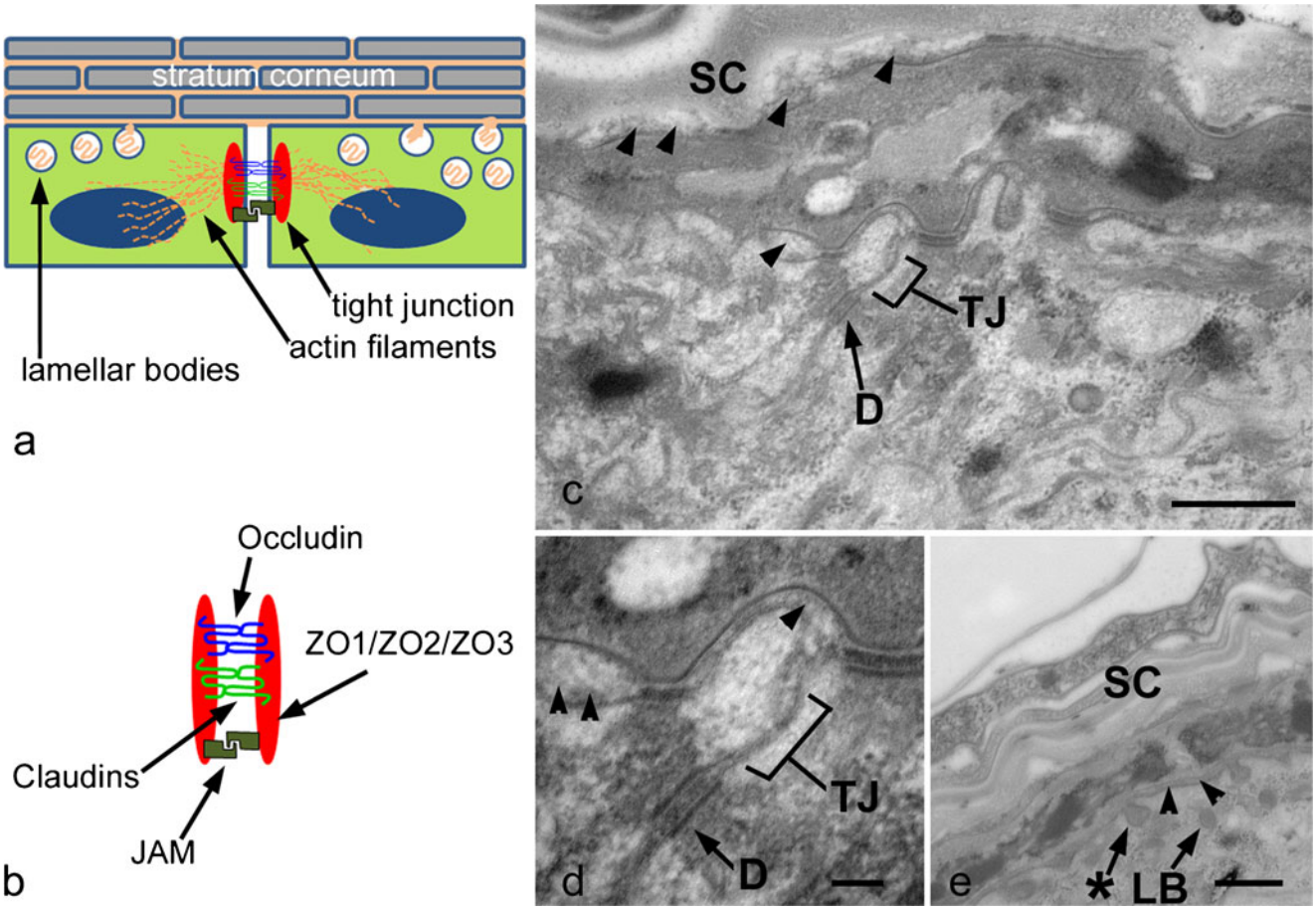

Fig. 2 Structural basis of the epidermal barrier. **a** Representation of the upper layer of the epidermis (granular layer and cornified layer) with lamellar bodies, lipid release to the surface and tight junctions that are attached to the actin cytoskleleton. **b** Representation of the structure of tight junctions and their components in skin (according to Proksch et al. 2008; Simpson et al. 2011). The epidermal barrier prevents evaporation (inside-out barrier) and penetration of microbes and chemicals (outside-in barrier). Penetration is counteracted by a series of features of the skin (acidic pH of the surface, the presence of commensals and antibacterial components and the continuous shedding of corneocytes). These features complement the cellular barrier (keratinocytes, which are able to respond to microbial contact by upregulating expression of antibacterial peptides and immunocompetent cells that reside in the epidermis between keratinocytes and that act as sentinels contributing to the antimicrobial barrier) and specialised structures such as the hydrophobic barrier created by the lipids that seal the spaces between the cornel scales in the cornified layer (stratum corneum) and the tight junctions of the granular layer (*JAM* junction adhesion molecule, *ZO* zonula occludens protein). Tight junctions and stratum corneum lipids not only provide a protection against penetration but also prevent evaporation (inside-out barrier). Adherens junctions and desmosomes (see Fig 3) participate in this function (Madison 2003; Brandner 2007; Proksch et al. 2008; Nestle et al. 2009; Kirschner et al. 2010; MacLeod and Havran 2011; Kirschner and Brandner 2012; Romani et al. 2012; Gallo and Nakatsuji 2011; Harder et al. 2013; Sugawara et al. 2013). **c–e** Transmission electron microscopy images of murine skin depicting corneal scales of the stratum corneum (*SC*) and the lipidic material deposited between the scales (*arrowheads*). The lipids are stored intracellularly in the form of lamellar bodies (**e**, *LB*) and released at the surface (**e**, *asterisk*) between the corneal scales (see also **a**; *TJ* tight junctions, *D* desmosomes). *Bars* 500 nm (**c**), 100 nm (**d**), 500 nm (**e**)

increase of basal TEWL (Sakai et al. 2003; Park et al. 2011). Whereas Sakai et al. (2003) concluded that the diabetic condition does not determine epidermal barrier injury, Park et al. (2011), in the type 2 rat model, reported additional structural alterations that might be indicative of a defective epidermal barrier. Specifically, they described a reduction in lamellar bodies and in lipids of the stratum corneum, with marked decreases in the synthesis of cholesterol and fatty acids and a corresponding decrease in expression for rate-limiting enzymes for epidermal lipid synthesis (Park et al. 2011). These findings were complemented by a decrease in antimicrobial peptides and delayed barrier recovery after tape stripping of the epidermis in the diabetic rats; both effects are more pronounced in animals with higher glycated haemoglobin (Park et al. 2011). These data suggest impairment of the epidermal barrier, damage that can even be present in the absence of increased basal TEWL.

Normal TEWL is not, indeed, synonymous with a healthy skin barrier. In contrast, TEWL is one of the parameters that can be measured to identify potential barrier disruptions (Proksch et al. 2008). Specifically, the superficial layers of

the epidermis not only prevent evaporation but also act as a barrier to penetration and, as such, perturbation of the latter might not be reflected in TEWL measurements (Proksch et al. 2008). Moreover, TEWL is not only dependent on barrier disruption but also on other factors such as gender, age, location, diurnal and seasonal variations (Black et al. 2000; Chilcott and Farrar 2000) and, most importantly, blood flow (Proksch et al. 2008), which is notoriously compromised in diabetic skin (Rendell et al. 2003; Schramm et al. 2006; Ngo et al. 2005; Dinh and Veves 2005). In other words, a healthy cutaneous blood flow is a necessary driving force to promote evaporation, which in turn is prevented or limited by the epidermal barrier. Therefore, in DM, even in the presence of a defective epidermal barrier, increased TEWL might not be manifest because of defective cutaneous circulation. For this reason, the lack of increased TEWL should not be taken as an absolute indicator for barrier function in DM.

Unaltered TEWL accompanied by structural defects in the barrier also occurs in senescent human skin. Here, combined alterations in sweating, cutaneous blood flow and temperature might affect TEWL, thus masking barrier defects, which are visible at a structural level (Ghadially et al. 1995). Likewise, in the Filaggrin null mouse, although TEWL is unaltered, a more permissive barrier to penetration is present (Kawasaki et al. 2012).

These considerations raise two fundamental questions: (1) whether, in human diabetic skin, other possible signs of a barrier defect might indeed exist, as is the case in the rat and (2) whether signs of barrier damage might be masked by the defective cutaneous microcirculation, as is the case in senescent skin.

Other important signs of barrier defect in OLETF rats include reduced levels of antimicrobial peptides (Park et al. 2011). In other diabetic models (db/db mouse with overt DM and HFD mouse), Taylor et al. (2011) reported defects in epidermal gamma-delta T cells. Given that gamma-delta T cells carry out their antimicrobial action at the front line of the epidermal barrier (Nestle et al. 2009; MacLeod and Havran 2011), defects in these cells might provide a further sign of barrier deficit. Finally, another important structural element of the epidermal barrier is the presence of functional tight junctions in the epidermis (Brandner 2007; Kirschner et al. 2010; Kirschner and Brandner 2012). Nothing is known about cutaneous tight junctions in DM. The fact that these structures are generally associated with the prevention of excessive evaporation (Brandner 2007), which has been reported not to occur in DM, might be a reason for the lack of interest shown in tight junctions in diabetic skin. However, as discussed above, unaltered TEWL in DM might not exclude other barrier defects and, to further complicate the scenario, the direct correlation between TEWL and epidermal barrier function has been questioned (Chilcott et al. 2002). Additionally, claudin-1 deficiency in mice is detrimental not only to epidermal tight junctions but also to the overall organisation of the upper epidermis (Sugawara et al. 2013). All these considerations suggest that the situation is intricate and that an assessment of cutaneous tight junctions in DM might add valuable elements to the overall discussion of the integrity of the epidermal barrier.

In summary, the functional status of the epidermal barrier in DM still needs to be fully clarified. Whereas a consensus seems to have been reached regarding the absence of excessive TEWL in DM, signs of barrier damage have been reported. Elucidation of the integrity of the epidermal barrier might improve our understanding of the risk of infection in DM.

Stability of junctional complexes and physical integrity of the epidermis in DM

The physical integrity of the epidermis is an important prerequisite for all skin functions. Junctional structures (Figs. 3, 4) connect keratinocytes with each other and to the basement membrane (BM), thus ensuring integrity and stability of the epidermal layer. Diabetic patients can experience a non-inflammatory blistering condition known as bullosis diabeticorum or diabetic bullae. These blisters occur spontaneously, i.e., without apparent trauma, mostly on the lower limbs and the separation can be intra- or subepidermal (Lipsky et al. 2000; Aye and Masson 2002; Ngo et al. 2005; Wohlrab et al. 2007; Bristow 2008; Larsen et al. 2008; Van Hattem et al. 2008; Behm et al. 2012). This condition has also been described in a pre-diabetic state (Lopez et al. 2009). Blistering in DM is generally considered to be rare, self-resolving and benign, affecting only about 0.5 % of diabetic patients (Aye and Masson 2002; Ngo et al. 2005; Wohlrab et al. 2007; Bristow 2008; Van Hattem et al. 2008; Ragunatha et al. 2011; Behm et al. 2012). However, despite this relatively small percentage, given the worldwide dimension of DM, diabetic bullae might nevertheless affect two and half million people by 2030. In any case, their rarity and harmlessness have been questioned, as severe consequences such as chronic and necrotic ulcerations have been reported (Lipsky et al. 2000; Larsen et al. 2008). Although the aetiology of this condition is unknown, a microvascular cause is suspected (Ngo et al. 2005). Alternatively, as reported by Bristow (2008), diabetic patients might intrinsically have lower epidermal stability. Cadherin disarray has been reported in diabetic mice (Taylor et al. 2011). Likewise, a reduction in the gene expression of type XVII and type IV collagen has been reported in normal skin of young db/db mice at a pre-diabetic or early diabetic stage (Rodgers et al. 2006). These two collagen types are typical components of hemidesmosomes and of the BM, respectively (Quondamatteo 2002; Gordon and Hahn 2010; van Agtmael and Bruckner-Tuderman 2010; Behrens et al. 2012). However, no studies have examined the fine structure of the cellular junctions and of the dermal epidermal junction

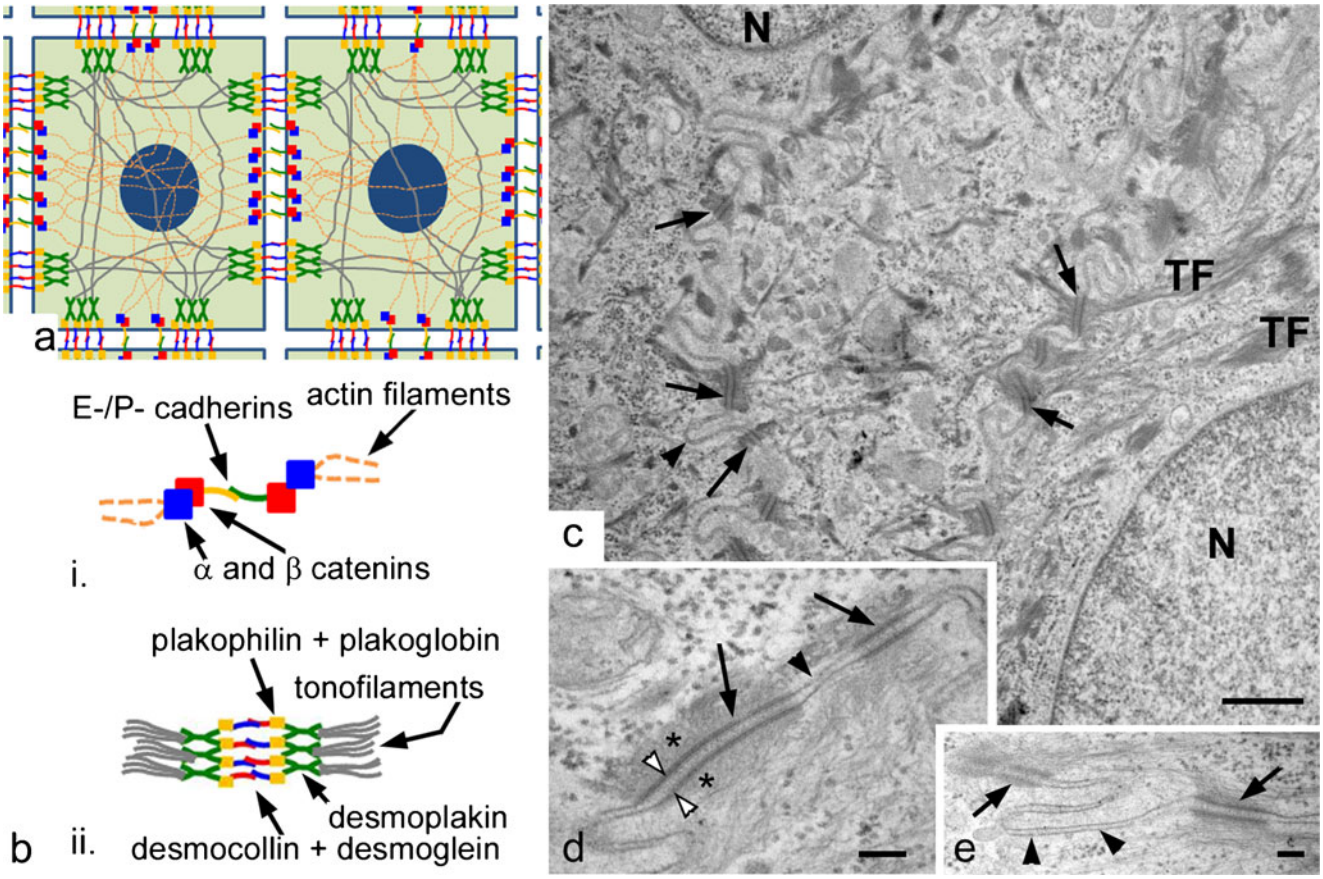

Fig. 3 Intercellular connections of the intermediate epidermal layers. **a** Representation of the intermediate epidermal layer (spinous layer). Mechanically stable intercellular connections are primarily provided by desmosomes linked intracellularly to the tonofilaments (*grey*). The cell membrane located between desmosomes contains adherens junctions that form a belt-like cell-cell junction connected intracellularly with the actin cytoskeleton (*orange dashed line*) that contribute to the maintenance of the epidermal structure (Vasioukhin et al. 2001; Simpson et al. 2011). **b** Representation of adherent junctions (*i*) and desmosomes (*ii*) and of their molecular components (according to Simpson et al. 2011). **c–e** Electron micrographs of murine skin showing cell-cell borders of keratinocytes (*N* nuclei of keratinocytes) from the intermediate layers of murine epidermis (equivalent of the spinous layer). Desmosomes are evident (*arrows*) and tonofilaments (*TF*) converge onto them forming an inner dense plaque (**d**, *asterisk*). The *darker line* near the inner dense plaque is the outer dense plaque (*open arrowheads*). According to the current model, this is the location at which the cytoplasmic domains of desmosomal cadherins (desmoglein and desmocollin) interact with desmoplakins via binding to plakophilin and plakoglobin (Desai et al. 2009). The portions of the membrane between two desmosomes are covered by adherent junctions (*solid arrowheads*). *Bars* 500 nm (**c**), 100 nm (**d**, **e**)

in diabetic skin as yet. Moreover, reduced deposition in heparan sulphate proteoglycan, i.e., another fundamental BM component (Quondamatteo 2002; Behrens et al. 2012), has been described in the epidermal BM of diabetic patients with nephropathy (van der Pijl et al. 1998). However, its depletion in the dermal-epidermal junction of diabetic patients has been reported to be more a result of the nephropathy itself rather than of the diabetic condition (van der Pijl et al. 1998). Interestingly, subepidermal blisters in diabetic patients are often associated with the occurrence of nephropathy (Ngo et al. 2005).

Indeed, some molecular signs related to structures contributing to epidermal stability have been described. However, whether corresponding structural defects that pre-dispose to a less stable epidermis also occur in DM is unknown.

Keratinocyte proliferation, epidermal thickness and keratinocyte differentiation in DM

As mentioned previously, neither OLETF rats (Park et al. 2011) nor STZ-treated mice (Sakai et al. 2003) show alterations in cutaneous filaggrin in their diabetic skin. The above studies have further examined additional differentiation markers such involucrin and loricrin immunohistochemically (Park et al. 2011) or keratin-1, -5 and -10 and loricrin by Western blotting (Sakai et al. 2003). Apart from some aberrant reactivity of the anti-loricrin antibodies with small peptides in the stratum corneum extracts of STZ-treated mice at 10 and 80 weeks (Sakai et al. 2003), neither study has found substantial alterations in such differentiation parameters (Sakai et al. 2003; Park et al. 2011).

In addition, Park et al. (2011) described normal keratinocyte proliferation and normal epidermal or dermal thickness in OLETF rats. In contrast, in two insulin-dependent models, i.e., STZ-treated mice (Sakai et al. 2003) and rats (Chen et al. 2010), a thinner epidermis has been measured. The authors also reported lower keratinocyte proliferation rates in diabetic mice in vivo (Sakai et al. 2003) as well as reduced proliferation in vitro when keratinocytes were exposed to glycation-modified albumin (Chen et al. 2010).

Similarly, thinner epidermis and reduced keratinocyte proliferation (plus poorly organised epidermis with fewer keratinocytes) have also been described in type 2 models together with a pathological distribution and a reduction in staining and in protein content of E-cadherin, and perturbations in keratin-5 and keratin-1. These include a pathological distribution and altered protein levels, namely an increase in keratin-1 and decrease in keratin-5, of which only the alteration of keratin-1 is statistically significant (Taylor et al. 2011). All these alterations described by Taylor et al. (2011) affect the db/db mouse at 12 weeks of age (i.e., at an age at which these mice are obese and have overt DM) and in HFD mice. Conversely, the lean non-diabetic controls or younger (6-week-old) db/db mice at an age at which DM is just starting to develop do not show such alterations (Taylor et al. 2011). An earlier study has reported increased gene expression for several keratin-associated proteins, and for keratin-2 and keratin-1 in db/db mice at 4–6 weeks of age (Rodgers et al. 2006). This stage corresponds to the young, pre- or early diabetic mice, investigated by Taylor et al. (2011), in which the amount of keratin-1 deposition is not altered. A possible explanation for this is that keratin-1 gene expression is upregulated at an early diabetic stage (Rodgers et al. 2006) and abnormal deposition becomes manifest later (Taylor et al. 2011). These data argue for defective keratinocyte differentiation in vivo in DM, at least in the db/db mouse.

In contrast, data on epidermal thickness and keratinocyte differentiation in diabetic human skin are scarce. Patients suffering from type 1 DM with various degrees of limited joint mobility showed a tendency to have a thicker epidermis on the dorsum of the hand, although statistically significant differences were only seen between controls and diabetic samples with a marked depletion of dermal hyaluronic acid (Bertheim et al. 2002). Conversely, a more recent study of patients who had well-controlled type 1 DM and in whom epidermal thickness was measured non-invasively, did not reveal any significant alteration (Zakharov et al. 2010).

Dermal extracellular matrix in DM

Most of the available knowledge on the dermal extracellular matrix (ECM; Fig. 5) in DM is based on collagen alterations and on the formation of advanced glycation endproducts (AGEs). These are pathological cross links that might originate from high glucose exposure and might or might not need oxidation to be formed in tissue. Collagen molecules are preferential targets for such modifications because of their slow turnover rates and high content in lysine residues (Paul and Bailey 1996; Wells-Knecht et al. 1997; Sell et al. 2005; Monnier et al. 2005; Avery and Bailey 2006; Monnier et al. 2008; Tessier 2010; Kennett et al. 2011).

As has been known for at least the past 20 years, glycation of collagen fibrils alters their organisation in vitro (Bai et al. 1992). More recently, a glycated collagen matrix has been shown to be more rigid, mechanically inferior and more brittle than a non-glycated collagen matrix (Liao et al. 2009). Accordingly, diabetic skin (*ex vivo*) is generally less mechanically stable when compared with its control counterpart, as shown in studies in human and db/db mice (Bermudez et al. 2011) and in TSOD mice (Ibuki et al. 2012).

Therefore, the general consensus is that the mechanical competence of the dermis is reduced in diabetes and that glycation of fibrillar collagen contributes to this reduction. Conversely, knowledge of the expression and deposition of dermal collagen in DM and therefore of its potential impact on the altered mechanical properties of diabetic skin is poorly defined.

Whereas in a baboon model of type 1 DM, no alteration of skin collagen organisation was found after X-ray diffraction analysis (Heffernan et al. 1996), several other groups have described various alterations in collagen in other examined animal models and in human skin. Depletion of type I procollagen in human leg skin has been reported in diabetic patients, both in the absence and in the presence of complications, with depletion being worse in patients with ulcers (Tahrani et al. 2012). Additionally, unlike for diabetic controls (i.e., patients with DM but no complications), significant disarray of the dermal collagen bundles has been reported after light microscopic analysis of the skin of patients with ulcers (Tahrani et al. 2012). A generally disorganised dermis has also been visualised by scanning electron microscopy of diabetic skin of 12-week-old TSOD mice with smaller and less dense fibres (Ibuki et al. 2012). These data, in addition to indicating pathological deposition, also indicate collagen reduction in diabetic skin. In agreement with this, a significant reduction in hydroxyproline content has been described in STZ-treated rats in which reduced collagen synthesis and reduced gene expression for type I collagen have also been reported (Craig et al. 1998). Similarly, decreased expression and production of dermal collagen has recently been described in AX-treated mice with overt DM and also in mice with blood glucose fluctuations (Ye et al. 2013). In contrast, with regard to type I collagen, Bermudez et al. (2011) described a decrease in content associated with increased gene expression in db/db mice. In their investigation, the decreased deposition

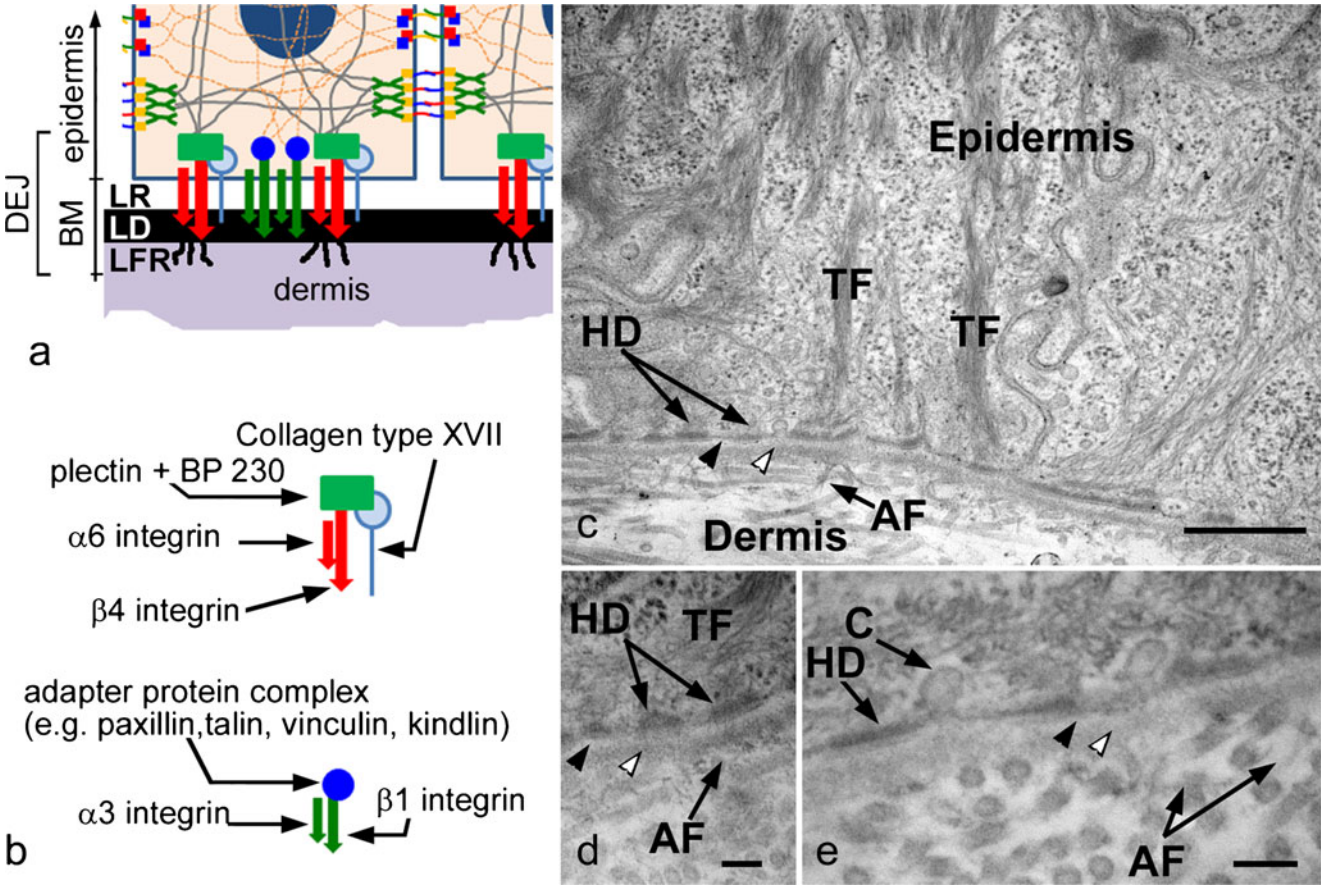

Fig. 4 Dermal epidermal junction (*DEJ*). **a**, **b** Representations of the DEJ (**a**) and of the molecular structure of its main components (**b**), according to Simpson et al. (2011). The epidermal basement membrane (*BM*) is the central structure of the DEJ. It has a lamina densa (*LD*), which is connected to the cellular layer via the lamina rara (*LR*) and to the underlying connective tissue via the lamina fibroreticularis (*LFR*). Adhesion of basal keratinocytes to the basement membrane is primarily ensured by hemidesmosomes (containing α6β4 integrins) and by sets of additional basement membrane receptors (e.g., β1 integrins) located in the basal aspect of the cell membrane of basal keratinocytes and between the hemidesmosomes. Hemidesmosomes are connected intracellularly to the tonofilaments (*grey lines*) and thus to desmosomes, whereas β1 integrins are connected intracellularly to the actin cytoskeleton (*orange dashed lines*) and thus to adherent junctions. For simplicity, other BM receptors (e.g., dystroglycan) have been omitted. The lower aspect of the BM is connected to the dermal collagen via anchoring fibrils (rich in type VII collagen), which form the LFR (Brakebusch et al. 2000; Vasioukhin et al. 2001; Krieg and Aumailley 2011; Desai et al. 2009; van Agtmael and Bruckner-Tuderman 2010; Simpson et al. 2011). **c–e** Electron micrographs of murine skin showing the DEJ at various levels of magnification (*HD* hemidesmosomes, *solid arrowheads* lamina rara of the epidermal basement membrane, *open arrowheads* lamina densa of the epidermal basement membrane, *AF* anchoring fibrils penetrating into the dermis and forming the lamina fibroreticularis, *TF* tonofilaments, *C* caveolae). *Bars* 500 nm (**c**), 100 nm (**d**, **e**)

of type I collagen is also accompanied by an increased deposition of type III collagen in diabetic skin, resulting in a reduced collagen I/III ratio (Bermudez et al. 2011). A lower I/III collagen ratio has been associated with reduced connective tissue stability, as indicated by studies on hernias (Klinge et al. 2006) and, in agreement with this, the presence of type III collagen has been associated with smaller collagen fibrils in developing tendon (Birk and Mayne 1997). In this context, the finding of Bermudez et al. (2011) of the reduced collagen I/III ratio might also contribute to explaining the lower mechanical stability of diabetic skin. This further suggests that the reduced mechanical competence of diabetic skin is not solely caused by collagen glycation and AGE formation. Additionally, the finding that a decrease in type I collagen content is associated with higher gene expression and lower matrix proteolysis (Bermudez et al. 2011) indicates that a primary defect in the deposition of collagen I occurs in DM. As a consequence of the reduced ECM deposition, proteolytic activity will be slowed or stopped and compensatory gene expression will be triggered. This scenario however will not apply to other studies that, in addition to a reduction of dermal collagen, have also described lower collagen I expression (Craig et al. 1998; Ye et al. 2013). Notably, the last-mentioned studies have involved insulin-dependent models, whereas Bermudez et al. (2011) investigated the db/db mouse, which is insulin-resistant. This raises the question as to whether differences in the homeostasis of collagens might exist between insulin-dependent and insulin-resistant DM.

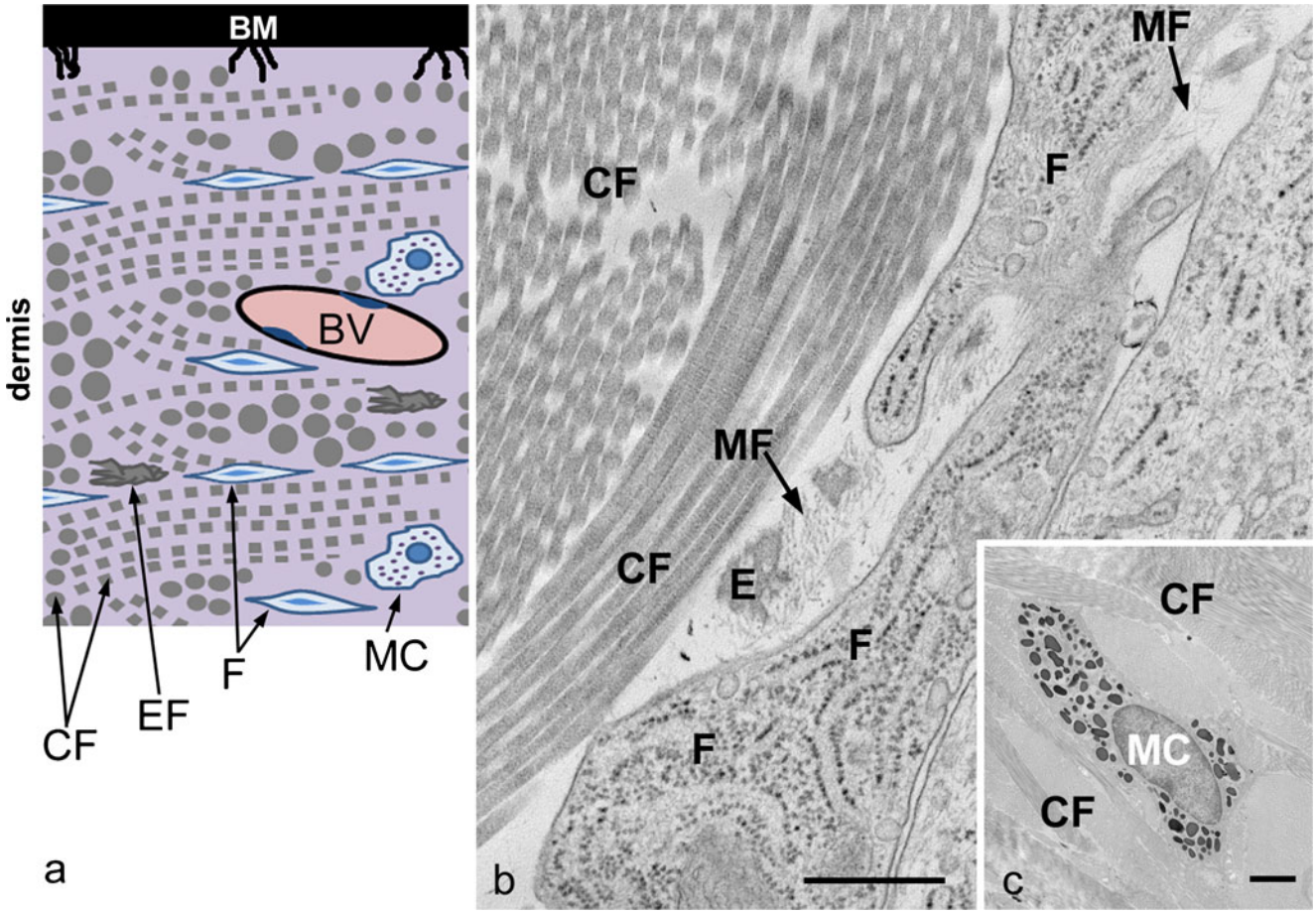

Fig. 5 Dermis. **a** Representation of an overview of the dermis after ultrastructural analysis by transmission electron microscopy. The extracellular space in the dermis is rich in collagen fibrils (*CF*). Most of the represented cells are fibroblasts (*F*). Other frequent extracellular structures are elastic fibres (*EF*) and microfibrils (not shown) possibly associated with elastic fibres or in a non-associated state (*BV* blood vessel, *MC* mast cell, *BM* epidermal basement membrane). **b**, **c** Electron micrographs of murine skin showing typical dermal structures (*F* fibroblasts, CF collagen fibrils, *MF* microfibrils, *E* amorphous elastin core of elastic fibres composed of elastin and flanked by MF). The microfibrils might also be non-associated to elastic fibres (*MC* mast cell). *Bars* 500 nm (**b**), 2 μm (**c**)

Bermudez et al. (2011) also described the increased expression of collagen III and V in the skin of the diabetic mouse. In another study on the same mouse model (db/db), Rodgers et al. (2006) noted reduced gene expression for various collagens, including I, III, V, VI and XIV (Rodgers et al. 2006). Notably, whereas the db/db mice studied by Rodgers et al. (2006) were aged 4–6 weeks, corresponding to an early or even pre-diabetic stage, the age group investigated by Bermudez et al. (2011), was 10–14 weeks, i.e., an age when the diabetic condition is obvious. This could explain some of the differences in gene expression seen in the two laboratories.

As mentioned above, Rodgers et al. (2006) also found reduced gene expression of type XIV collagen. This is a fibril-associated collagen (Krieg and Aumailley 2011; Gordon and Hahn 2010) thought to participate in the control of the size of collagen fibrils by preventing the fusion of fibrils (Gordon and Hahn 2010). Therefore, a drop in the expression of a negative regulator of collagen fibre thickness (such as type XIV collagen) would seem to contradict the presence of thin fragile collagen fibres as described in the diabetic TSOD mouse (Ibuki et al. 2012). This might further complicate the issue of the regulation of collagen deposition and expression in diabetic skin.

Taken together, although AGEs surely play a prominent role in the collagen damage in the diabetic skin, this body of literature suggests that the deregulation of mechanisms of transcription and the deposition of fibrillar collagens in the dermal matrix also contribute to the poor mechanical competence of skin in DM.

The effects of AGE formation on the ECM however go beyond alterations of a biomechanical nature. Cross links such as glucosepane might also involve arginine residues and these latter could be part of the RGD (arginine—glycine—aspartic acid) or GFOGER (glycine—phenylanaline—hydrohyproline—glycine—glutamic acid—arginine) domains of ECM proteins (Monnier et al. 2005; Sell et al. 2005; Avery and Bailey 2006; Monnier et al. 2008). Given that such residues are known

ECM protein sequences recognised by integrins (Leitinger and Hohenester 2007; Barczyk et al. 2010), their alteration might interfere with ECM/integrin interactions and, ultimately, with the biological effects of dermal ECM. This obviously opens a new avenue of interest in the study of the dermal matrix in DM. A notion that has become increasingly obvious in the ECM field over the past 20 years is that the biological function of the ECM depends more on the overall mixture and interactions of matrix components and thus on the suprastructure of the matrix, rather than on the individual and possibly more abundant components (Bruckner 2010). Hence, molecules considered to be "minor" might be of crucial importance and might be determinants of differential functions between two otherwise similar matrices (Bruckner 2010). For a comprehensive understanding of the biological role of the ECM in a given compartment (e.g., the dermis), investigations of its detailed composition are therefore fundamental. This becomes even more relevant in the dermal ECM in diabetes, given that the diabetic condition is able to interfere with the ECM-integrins interactions and thus with the biological action of the dermal ECM. Therefore, a thorough understanding of the dermal matrix in diabetes should deliver answers regarding its possible pathogenetic role in skin damage in DM.

Additionally, AGEs, other than having the potential for interfering with the ECM, possess intrinsic biological activity by acting on cellular receptors (Schmidt et al. 1992; Brett et al. 1993; Fujimoto et al. 2010; Zhu et al. 2011). Thus, the glycated matrix can have an impact on dermal fibroblasts (Liao et al. 2009; Pageon 2010) and keratinocytes (Fujimoto et al. 2010; Zhu et al. 2011). Interestingly, both the studies of Fujimoto et al. (2010) and Zhu et al. (2011) refer to an AGE-dependent increase in matrix metalloproteinase-9 (MMP-9) in human keratinocytes, which in vivo is reported to be significantly decreased in quantity in human diabetic skin by some authors (Bermudez et al. 2011) or unaltered in quantity but more active by others (Tahrani et al. 2012).

In general, MMPs play a prominent role in controlling ECM remodelling and, in this way, these enzymes also control the composition and therefore the function, of the ECM (Lu et al. 2011). At least six studies have addressed MMPs in diabetic skin in vivo. In most of the cases, a higher proteolytic status has been described in human skin (Tahrani et al. 2012), in STZ-treated rats (Takahashi and Takasu 2011), in AX-treated mice (Ye et al. 2013) and in the subcutis of TSOD mice (Ibuki et al. 2012). Ye et al. (2013) also showed that the higher proteolytic status attributable to DM is exacerbated in cases of blood glucose fluctuations (Ye et al. 2013). In contrast, in db/db mice of a young age (early DM or pre-disease), no changes in the expression of genes relevant to ECM remodelling have been found (Rodgers et al. 2006), whereas in older db/db mice (notably diabetic), a net decrease in proteolytic activity has been reported (Bermudez et al. 2011).

Another important component of the human dermal matrix, namely hyaluronic acid, has been studied in human skin in patients affected by insulin-dependent DM. A considerable reduction in hyaluronic acid, particularly in the region of the dermal epidermal junction, has been found in the dermis of patients with low joint mobility, whereas in patients with little or no impairment of joint mobility, hyaluronic acid distribution predominantly resembles that of the normal condition (Bertheim et al. 2002). Interestingly, an increase in hyaluronidase has been described in the skin of STZ-treated rats (Takahashi and Takasu 2011).

Taken together, neither the regulation of collagen synthesis and production, nor matrix remodelling seems to be uniform in individual reports on diabetic skin in vivo. Additional factors that differentiate the DM types and the various models might have an influence on collagen homeostasis.

Dermal microvessels and their BM in diabetic skin

Similar to the retina, glomeruli and endoneurium, the skin in DM is affected by microangiopathy with defective vasodilation which is exacerbated by neuropathy (Rendell et al. 2003; Schramm et al. 2006; Ngo et al. 2005; Dinh and Veves 2005). This can result in poor skin nutrition and is believed to contribute to a number of cutaneous injuries, including the poor regeneration potential of diabetic skin (Falanga 2005; Ngo et al. 2005). On a structural note, similarly to the other diabetic microangiopathy sites, thickened microvascular BMs have also been reported in skin (Banson and Lacy 1964; Moore and Frew 1965; Braverman and Keh-Yen 1984; Braverman et al. 1990; Dinh and Veves 2005; Ngo et al. 2005; Schramm et al. 2006; Kiliçaslan and Özer 2009; Chen et al. 2010). Such microvascular alterations have been proposed to be the hallmark of accelerated aging of diabetic skin (Braverman and Keh-Yen 1984). At the same time, such microvascular thickening is thought to be detrimental to vasodilation, nutrient diffusion and leucocyte migration and might account for impaired wound closure and increased wound infection (Dinh and Veves 2005; Schramm et al. 2006). Despite this concept, a closer association between BM thickening and microvascular dysfunction has yet to be made. For example, nothing is known regarding possible alterations in the composition of the BMs in cutaneous microvessels, alterations that might, in turn, account for their dysfunction in DM. In this context, the db/db mouse, which presents several characteristics of type 2 DM, including delayed wound healing (Greenhalgh et al. 1990; Kobayashi et al. 2000) and which is widely used in diabetic wound healing studies (Greenhalgh et al. 1990; Rodgers et al. 2006; Berdal et al. 2011; Bermudez et al. 2011; Zhao et al. 2012), might represent a useful

comparison. However, the cutaneous microvascular structural phenotype of this mouse model has never been studied. Indeed, addressing this aspect should add valuable data in understanding the correlation between potential structural and/or molecular aberrations in skin microvessels and poor wound healing in DM.

Another important component of the microvascular wall is represented by the pericytes. Upon injury, these cells interact with endothelial cells, connective tissue and infiltrating inflammatory cells and are, thus, a central point of interchange of various signals during tissue repair (Dulmovits and Herman 2012). Therefore, perturbations in the pericytes of skin microvessels might affect wound healing potential. Pericyte loss in microvessels of diabetic skin has been reported in the literature (Dulmovits and Herman 2012) together with morphological alterations related to the excessive deposition of BM material in the microvascular wall (Braverman et al. 1990). The number of contact points between endothelial cells and pericytes is markedly diminished in DM and this enhances the physical separation between the two cell types. Hence, in diabetic pericytes, cell protrusions trying to make contact with endothelial cells are thinner and longer (Braverman et al. 1990). This renders the pericyte/endothelial interactions more difficult and might negatively impact on pericyte functionality, thus contributing to microvascular dysfunction in DM and especially in wound healing.

Reports differ regarding the density and size of cutaneous microvessels in DM (Algenstaedt et al. 2003; Ngo et al. 2005; Schramm et al. 2006; Aellen et al. 2012; Tellechea et al. 2013). Moreover, the specific impact of hyperglycaemia on such parameters is not completely clear. Reduced capillary density has, indeed, been described both in correlation to high levels of glycaemia and in pre-diabetes in the same mouse model of type 2 DM (Algenstaedt et al. 2003; Schaefer et al. 2010). The same studies describe a general increase in capillary size possibly attributable to a loss of smaller vessels (Algenstaedt et al. 2003; Schaefer et al. 2010). A recent investigation of skin samples of patients and of three different animal models of insulin-dependent DM found an increased vessel density in all cases (Tellechea et al. 2013). Sangiorgi et al. (2010) showed vascular regression, disarray, disruption and occlusion in microvessels of diabetic skin obtained from an amputation, i.e., most likely from poorly controlled DM. In contrast, an absence of alterations in skin capillary density in vivo has been reported in diabetic patients with well-controlled hypertension (Aellen et al. 2012). However, diabetic patients with higher glycated haemoglobin have a lower microvascular density (Aellen et al. 2012). This supports the idea that higher glycaemia can indeed contribute to the worsening of the cutaneous microcirculation in DM. Interestingly, in young db/db mice (i.e., at a pre- or early diabetic stage), gene expression of type XV collagen is increased (Rodgers et al. 2006). Collagen XV is one of the possible progenitors of the anti-angiogenic molecule endostatin (Sasaki et al. 2000; Gordon and Hahn 2010). Whether this plays a role in the overall scenario of poor vascularisation is unknown. At the same time, this and/or other similar, yet unidentified, features might contribute to the entire microvascular deficit, and be further pathogenetic factors in addition to the accumulation of AGE in the vascular wall. Moreover, knowledge of dermal microvascular BM in DM (beyond thickening) is scarce and the potential impact of BM alterations in compromising microvascular function remains theoretical. Deeper comprehension of the structure of the microvascular BM and of the composition of dermal capillaries in DM will contribute to a better understanding of cutaneous microangiopathy and will further add to the clarification of the pathogenesis of poor wound healing and other cutaneous manifestations caused by poor skin nutrition.

Outlook

Given its relatively easy accessibility, both for taking small biopsies and for non-invasive sampling, the skin has an enormous diagnostic potential in providing biomarkers for DM. Skin biopsies have some limitations, because of patient recovery (Paliwal et al. 2013), which can be critical in DM. Despite this, biopsy-based studies in diabetic patients can be successfully carried out under well-controlled conditions (Tahrani et al. 2012; Tellechea et al. 2013) demonstrating the feasibility of this approach.

Biomarkers in DM are scarce. One of the major candidates is the non-fluorescent AGE glucosepane (Monnier et al. 2005; Sell et al. 2005). Although it has recently been associated with long-term complications in type 1 DM (Monnier et al. 2013), an understanding of its full potential and its possible pathogenetic role in skin damage in DM is still in its infancy. Another recent study has related the depletion of cutaneous procollagen I specifically to DM, with this being more marked in patients with foot ulcers than in non-complicated DM (Tahrani et al. 2012). As a further biopsy-based tool, the study of skin fibroblasts is being evaluated for monitoring type 1 DM (Millioni et al. 2012).

In order to lessen patient burden, especially in those patients who need several daily checks, non-invasive approaches are presently under development (for details, see Kottman et al. 2012; Sivanandam et al. 2012; Vashist 2012; Mayrovitz et al. 2013; Pleitez et al. 2013) or, as for skin autofluorescence (SAF), have been intensively evaluated (e.g., Lyons et al. 1991; Monnier et al. 1999; Maynard et al. 2007; Gerrits et al. 2008a, 2008b; Stirban et al. 2008; Ghazaryan et al. 2012; Tanaka et al. 2012; Noordzij et al.

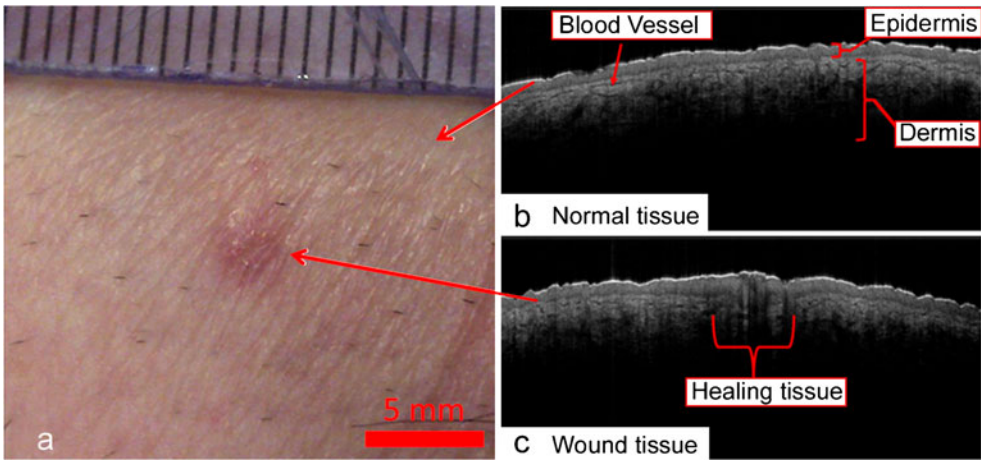

Fig. 6 OCT scan of a pin-prick wound of the forearm. **a** Pin-prick wound on the dorsal aspect of a forearm. **b** Structural OCT B-Scan of the non-wounded site. **c** Structural OCT B-Scan of the wound site. The images were kindly provided by Prof. Martin Leahy, Physics, NUI Galway

2012; Mascai et al. 2013). A fundamental limitation of these methods is the signal:noise ratio attributable to the biophysical, molecular and structural characteristics of the skin (Mulder et al. 2006; Vashist 2012). SAF for example has recently been successfully used to mirror the diabetic state in a well-controlled patient cohort after exclusion of potential interfering factors, e.g., smoking or kidney failure (Mascai et al. 2013). Otherwise, SAF appears to be an indicator for cumulative hyperglycaemic damage and a complication predictor, rather than a specific index of the glycaemia (Lyons and Basu 2012).

Finally, the skin is notably the gateway to the body for a number of non-invasive imaging technologies (for a review, see Daly and Leahy 2013). These depend on the passage of signals through the cutaneous layers and, therefore, the biochemical/biophysical features of skin components might theoretically interfere with them and produce artifacts. For example, optical coherence tomography (OCT), which is

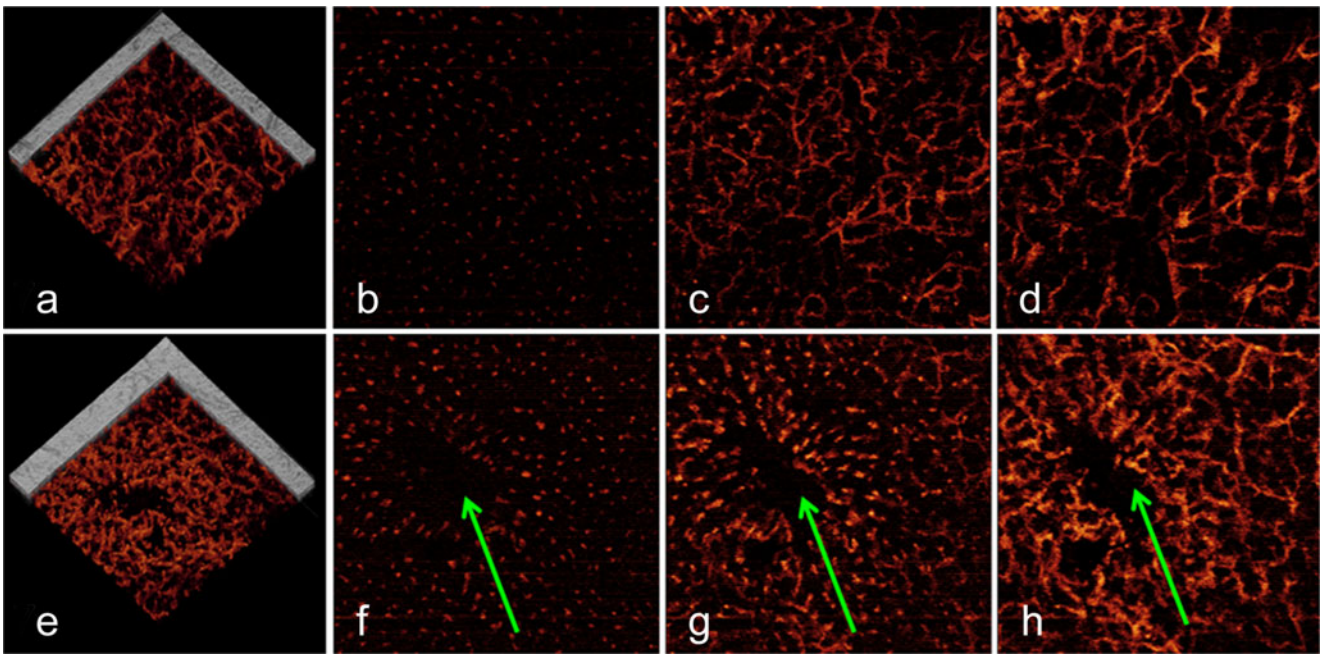

Fig. 7 Skin plexus and its visualisation by means of cm-OCT of skin at various levels. **a–d** Normal skin. **e–h** Pin-prick wound. This technique allows to image movement and to exclude the structure at the same time. Therefore, all the structural parts of the tissue, with the exception of the blood vessels (*red* to *orange*) that contain cells in movement, can be computationally blanked out. Furthermore, it is possible to scan and slice the tissue at various levels. **a**, **e** A three-dimensional view of the vascular plexus. The epidermis (still visible at the margin of the field) was otherwise excluded, as was the case for the rest of the skin structures, with the exception of the vascular profile. **b**, **f** Relatively superficial slices of the papillary region of the dermis in which the tips of the blood vessels are visible in the papillae (*red*/*orange dots*). The remaining images show deeper slices at the level of the superficial plexus, which is located between the bottom region of the papillary dermis and the top region of the reticular dermis. Note the nearly horizontal course of the vessels. The slices in **d**, **h** are at approximately 200–300 μm from the level of those in **b**, **f**, whereas those in **c**, **d** are at an intermediate level (*green arrow* prick wound plexus). The images were kindly provided by Prof. Martin Leahy, Physics, NUI Galway

based on skin penetration by optic signals and on the detection of reflected and backscattered light (Marschall et al. 2011), has shown that the presence of gel or glycerol, instead of air, between the light source and the skin surface improves the visualisation of blood vessels (Liew et al. 2011). Similar results have been obtained by using a skin phantom (Liew et al. 2011). This means that changes in the layers crossed by the optic signals can affect the clarity of the recording from the deeper regions (i.e., the vessels). OCT and its variants are strongly emerging in dermatological research (Daly and Leahy 2013; Enfield et al. 2011; Zafar et al. 2013; Zam et al. 2013), enabling visualisation of the epidermis with a distinct dermal epidermal junction (see Fig. 6). A variant of this developed by Martin Leahy and his group (correlation mapping OCT, cm-OCT) enables, via a similar principle, the mapping of blood vessels in the skin at various levels of tissue depth (Enfield et al. 2011; Zafar et al. 2013; Zam et al. 2013, see also Fig. 7). The use of these non-invasive diagnostic tools will presumably increase in the near future. Since structural/molecular changes in skin can interfere with optical signals and pose a serious limitation to their use, this issue should be addressed for the correct interpretation of the readings. In the case of DM in which skin alterations are still poorly defined, a precise understanding of the actual texture and composition of diabetic skin will contribute to overcome such limitations, thereby supporting and promoting the use of non-invasive diagnostics in diabetic patients.

Concluding remarks

Interest is increasing in the structure and molecular composition of non-injured diabetic skin, as shown by the fact that a large part of the available knowledge is relatively recent. Although skin alterations clearly occur in DM, the overall picture is not uniform. A consensus seems to have been attained regarding some features (e.g., diabetic skin is mechanically less competent), whereas others (e.g., the epidermal barrier) are still unclear. Underlying pathogenetic mechanisms have not been fully elucidated. Further open questions include the impact of hyperglycaemia (severity or duration) or of animal models. Addressing the above issues should help to identify potential markers or therapeutic targets for the prevention and monitoring of DM and of its complications. Finally, a full understanding of skin structure in DM is crucial for the interpretation of non-invasive deep-tissue diagnostics in which optic signals crossing skin layers might be distorted by altered tissue texture or composition.

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References

Aellen J, Dabiri A, Heim A, Liaudet L, Burnier M, Ruiz J, Feihl F, Waeber B (2012) Preserved capillary density of dorsal finger skin in treated hypertensive patients with or without type 2 diabetes. Microcirculation 19:554–562

Algenstaedt P, Schaefer C, Biermann T, Hamann A, Schwazloh B, Greten H, Ruther W, Hansen-Algenstaedt N (2003) Microvascular alterations in diabetic mice correlate with level of hyperglycemia. Diabetes 52:542–549

American Diabetes Association (2013) Diagnosis and classification of diabetes mellitus. Diabetes Care 36:S67–S74

Avery NC, Bailey AJ (2006) The effects of the Maillard reaction on the physical properties and cell interactions of collagen. Pathol Biol 54: 387–395

Aye M, Masson EA (2002) Dermatological care of the diabetic foot. Am J Clin Dermatol 3:463–474

Bai P, Phua K, Hardt T, Cernadas M, Brodsky B (1992) Glycation alters collagen fibril organisation. Connect Tissue Res 28:1–12

Banson BB, Lacy PE (1964) Diabetic microangiopathy in human toes with emphasis on the ultrastructural change in dermal capillaries. Am J Pathol 45:41–58

Barczyk M, Carracedo S, Gullberg D (2010) Integrins. Cell Tissue Res 339:269–280

Behm B, Schreml S, Landthaler M, Babilas P (2012) Skin signs in diabetes mellitus. J Eur Acad Dermatol Venereol 26:1203–1211

Behrens DT, Villone D, Koch M, Brunner G, Sorokin L, Robenek H, Bruckner-Tuderman L, Bruckner P, Hansen U (2012) The epidermal basement membrane is a composite of separate laminin- or collagen IV-containing networks connected by aggregated perlecan, but not by nidogens. J Biol Chem 287:18700–18709

Berdal M, Appelbom HI, Eikrem JH, Lund A, Busund L, Hanes R, Seljelid R, Jenssen T (2011) Aminated β-1,3-D-glucan has a dose-dependent effect on wound healing in diabetic *db*/*db* mice. Wound Rep Reg 19:579–587

Bermudez DM, Herdrich BJ, Xu J, Lind R, Beason DP, Mitchell ME, Soslowsky LJ, Liechty KW (2011) Impaired biomechanical properties of diabetic skin. Am J Pathol 178:2215–2222

Bertheim U, Engstorm-Laurent A, Hofer P, Hallgren P, Asplund J, Hellstrom S (2002) Loss of hyaluronan in the basement membrane zone of the skin correlates to the degree of stiff hands in diabetic patients. Acta Derm Venereol 82:329–334

Birk D, Mayne R (1997) Localization of collagen types I, III and V during tendon development. Changes in collagen types I and III are correlated with changes in fibril diameter. Eur J Cell Biol 72:352–361

Black D, Del Pozo A, Lagarde JM, Gall Y (2000) Seasonal variability in the biophysical properties of stratum corneum from different anatomical sites. Skin Res Technol 6:70–76

Brakebusch C, Grose R, Quondamatteo F, Ramirez A, Jorcano JJ, Pirro A, Svensson M, Herken R, Sasaki T, Timpl R, Werner S, Fässler R (2000) Skin and hair follicle integrity is crucially dependent on β1 integrin expression on keratinocytes. EMBO J 19:3990–4003

Brandner JM (2007) Pores in the epidermis: aquaporins and tight junctions. Int J Cosmet Sci 29:413–422

Braverman IM, Keh-Yen A (1984) Ultrastructural abnormalities of the microvasculature and elastic fibres in the skin of juvenile diabetics. J Invest Dermatol 82:270–274

Braverman IM, Sibley J, Keh-Yen A (1990) Ultrastructural analysis of the endothelial-pericyte relationships in diabetic cutaneous vessels. J Invest Dermatol 95:147–153

Brett J, Schmidt AM, Yan SD, Weidman E, Pinsky D, Nowygrod R, Neeper M, Przysiecki C, Shaw A, Migheli A, Stern D (1993) Survey of the distribution of a newly characterised receptor for advanced glycation end products in tissues. Am J Pathol 143:1699–1712

Bristow I (2008) Non-ulcerative skin pathologies of diabetic foot. Diabetes Metab Res Rev 24:S84–S89

Bruckner P (2010) Suprastructures of extracellular matrices: paradigms of functions controlled by aggregates rather than molecules. Cell Tissue Res 339:7–18

Buckley CM, O'Farrell A, Canavan RJ, Lynch AD, De La Harpe DV, Bradley CP, Perry IJ (2012) Trends in the incidence of lower extremity amputations in people with and without diabetes over five-year period in the Republic of Ireland. PLOS ONE 7:e41492

Chatzizgeorgiou A, Halapas A, Kalafatakis K, Kamper E (2009) The use of animal models in the study of diabetes mellitus. In Vivo 23:245–258

Chen X, Lin W, Lu S, Xie T, Kui G, Shi Y, Zou J, Liu Z, Liao W (2010) Mechanisitic study of endogenous skin lesions in diabetic rats. Exp Dermatol 19:1088–1095

Chilcott RP, Farrar R (2000) Biophysical measurements of human forearm skin in vivo: effects of site, gender, chirality, and time. Skin Res Technol 6:64–69

Chilcott RP, Dalton CH, Emmanuel AJ, Allen CE, Bradley ST (2002) Transepidermal water loss does not correlate with skin barrier function in vitro. J Invest Dermatol 118:871–875

Craig RG, Yu Z, Xu L, Barr R, Ramamurthy N, Boland J, Schneir M, Golub LM (1998) A chemically modified tetracycline inhibits streptozotocin-induced diabetic depression of collagen synthesis and steady-state type I procollagen mRNA. Biochim Biophys Acta 1402:250–260

Daly SM, Leahy MJ (2013) "Go with the flow": a review of methods and advances in blood flow imaging. J Biophotonics 6:217–255

Desai BV, Harmon RM, Green KJ (2009) Desmosomes at a glance. J Cell Sci 122:4401–4407

Dinh TL, Veves A (2005) A review of the mechanisms implicated in the pathogenesis of the diabetic foot. Int J Low Extrem Wounds 4:154–159

Dulmovits BM, Herman IM (2012) Microvascular remodelling and wound healing: a role for pericytes. Int J Biochem Cell Biol 44:1800–1812

Eaglstein WH, Callen JP (2009) Dermatologic comorbidities of diabetes mellitus and related issues. Arch Dermatol 145:467–469

Enfield J, Jonathan E, Leahy M (2011) In vivo imaging of the microcirculation of the volar forearm using correlation mapping optical coherence tomography (cmOCT). Biomed Opt Exp 2:1184–1193

Falanga V (2005) Wound healing and its impairment in the diabetic foot. Lancet 366:1736–1743

Fujimoto E, Kobayashi T, Fujimoto N, Akiyama M, Tajima S, Nagai R (2010) AGE-modified collagens I and III induce keratinocyte terminal differentiation through AGE receptor CD36: epidermal-dermal interaction in acquired perforating dermatosis. J Invest Dermatol 130:405–414

Gallo RL, Nakatsuji T (2011) Microbial symbiosis with the innate defense system of the skin. J Invest Dermatol 131:1974–1980

Geerlings SE, Stolk RP, Camps MJL, Netten PM, Hoekstra JBL, Bouter KP, Bravenboer B, Collet JT, Jansz AR, Hoepelman ALM (2000) Asymptomatic bacteriuria may be considered a complication in women with diabetes. Diabetes Care 23:744–749

Gerrits EG, Lutgers HL, Kleefstra N, Graaff R, Groenier KH, Smit AJ, Gans RO, Bilo HJ (2008a) Skin autofluorescence: a tool to identify type 2 diabetic patients at risk for developing microvascular complications. Diabetes Care 31:517–520

Gerrits EG, Lutgers HL, Kleefstra N, Groenier KH, Smit AJ, Gans RO, Bilo HJG (2008b) Skin advanced glycation end product accumulation is poorly reflected by glycemic control in type 2 diabetic patients (ZODIAC-9). J Diabetes Sci Technol 2:572–577

Ghadially R, Brown BE, Sequiera-Martin SM, Feingold K, Elias PM (1995) The aged epidermal permeability barrier: structural, functional, and lipid biochemical abnormalities in human and a senescent murine model. J Clin Invest 95:2281–2290

Ghazaryan AA, Hu P-S, Chen S-J, Tan H-Y, Dong C-Y (2012) Spatial and temporal analysis of skin glycation by the use of multiphoton microscopy and spectroscopy. J Dermatol Sci 65:189–195

Gordon MK, Hahn RA (2010) Collagens. Cell Tissue Res 339:247–257

Greenhalgh DG, Sprugel KH, Murray MJ, Ross R (1990) PDGF and FGF stimulate wound healing in the genetically diabetic mouse. Am J Pathol 136:1235–1246

Hamilton E, Martin N, Makepeace A, Sillars BA, Davis WA, Davis TME (2013) Incidence and predictors of hospitalization for bacterial infection in community-based patients with type 2 diabetes: the Freemantle diabetes study. PLOS ONE 8:e60502

Harder J, Schröder J-M, Gläser R (2013) The skin surface as antimicrobial barrier: present concepts and future outlooks. Exp Dermatol 22:1–5

Heffernan S, James V, Zilkens R, Kirwan P, Birrell A, McLennan S, Hennessy A, Gillin A, Horvath J, Tiller D, Yue D, Turtle J (1996) Changes of extracellular matrix in a baboon (*Papio hadryas*) model of insulin dependent diabetes: studies using electron microscopy and X-ray diffraction techniques. Diabetes Res Clin Pract 34:65–72

Hirayama I, Yi Z, Izumi S, Arai I, Suzuki W, Nagamachi Y, Kuwano H, Takeuchi, Izumi T (1999) Genetic analysis of obese diabetes in the TSOD mouse. Diabetes 48:1183–1191

Ibuki A, Akase T, Nagase T, Minematsu T, Nakagami G, Horii M, Sagara H, Komeda T, Kobayashi M, Shimada T, Aburada M, Yoshimura K, Sugama J, Sanada H (2012) Skin fragility on obese mice: possible involvement of elevated oxidative stress upregulation of matrix metalloproteinases. Exp Dermatol 21:178–183

Iizuka S, Suzuki W, Tabuchi M, Nagata M, Imamura S, Kobayashi Y, Kanitani M, Yanagisawa T, Kase Y, Takeda S, Aburada M, Takahashi KW (2005) Diabetic complications in a new animal model (TSOD mouse) of spontaneous NIDDM with obesity. Exp Anim 54:71–83

Jackson LA (2005) Evaluating diabetes mellitus as a risk factor for community-acquired infections. Clin Infect Dis 41:289–290

Kawasaki H, Nagao K, Kubo A, Hata T, Shimizu A, Mizuno H, Yamada T, Amagai M (2012) Altered stratum corneum barrier and enhanced percutaneous immune response in filaggrin-null mice. J Allergy Clin Immunol 129:1538–1546

Kennett EC, Chuang CY, Degendorfer G, Whitekock JM, Davies MJ (2011) Mechanisms and consequences of oxidative

damage to extracellular matrix. Biochem Soc Trans 39:1279–1287
Kiliçaslan SMS, Özer C (2009) Effects of benfluorex—vitamin C supplementation on cutaneous capillaries of diabetic rats. Cell Biol Int 33:442–446
King AJ (2012) The use of animal models in diabetes research. Br J Pharm 166:877–894
Kirschner N, Brandner JM (2012) Barriers and more: functions of tight junction proteins in the skin. Ann N Y Acad Sci 1257:158–166
Kirschner N, Houdek P, Fromm M, Moll I, Brandner JM (2010) Tight junctions form a barrier in human epidermis. Eur J Cell Biol 89:839–842
Klinge U, Binnebösel M, Mertens PR (2006) Are collagens the culprit in the development of incisional and inguinal hernia diseases? Hernia 10:472–477
Knapp S (2012) Diabetes and infection: is there a link? A mini review. Gerontology 26:1–6
Kobayashi K, Forte TM, Taniguchi S, Ishida B, Oka K, Chan L (2000) The db/db a model for diabetic dyslipidemia: molecular charectarisation and effects of western diet feeding. Metabolism 49:22–31
Koivukangas V, Annala AP, Salmela PI, Oikarinet A (1999) Delayed restoration of epidermal barrier function after blister injury in patients with diabetes mellitus. Diabet Med 16:563–567
Kottman J, Rey JM, Luginbuhl J, Reichmann E, Sigrist MW (2012) Glucose sensing in human epidermis using mid-infrared photoacoustic detection. Biomed Opt Expr 3:667–680
Krieg T, Aumailley M (2011) The extracellular matrix of the dermis: flexible structures with dynamic functions. Exp Dermatol 20:689–695
Kudo T, Shimada T, Toda T, Igeta S, Suzuki W, Ikarashi N, Ochiai W, Itoa K, Aburada M, Sugiyama K (2009) Altered expression of CYP in TSOD mice: a model of type 2 diabetes and obesity. Xenobiotica 39:889–902
Lam DW, LeRoith D (2012) The worldwide diabetes epidemic. Curr Opin Endocrinol Diabetes Obes 19:93–96
Larsen K, Jensen T, Karlsmark T, Holstein PE (2008) Incidence of bullosis diabeticorum—a controversial cause of chronic foot ulceration. Int Wound J 5:591–596
Leitinger B, Hohenester E (2007) Mammalian collagen receptors. Matrix Biol 26:146–155
Liao H, Zakhaleva J, Chen W (2009) Cells and tissue interactions with glycated collagen and their relevance to delayed diabetic wound healing. Biomaterials 30:1689–1696
Liew YM, McLaughlin RA, Wood FM, Sampson DD (2011) Reduction of image artifacts in three-dimensional optical coherence tomography of skin in vivo. J Biomed Opt 16:116018
Lipsky BA, Baker PD, Ahroni JH (2000) Diabetic bullae: 12 cases of purportedly rare cutaneous disorder. Int J Dermatol 39:196–200
Lipsky BA, Itani KMF, Weigelt JA, Joseph W, Paap CM, Reisman A, Myers DE, Huang DB (2011) The role of diabetes mellitus in the treatment of skin and skin structure infections caused by methicillin-resistant *Saphylococcus aureus*: results from three randomised controlled trials. Int J Infect Dis 15:e140–e146
Lopez PR, Leicht S, Sigmon JR, Stigall L (2009) Bullosis diabeticorum associated with a prediabetic state. South Med J 102:643–644
Lowell BB, S-Susulic V, Hamann A, Lawitts JA, Himms-Hagen J, Boyer BB, Kozak LP, Flier JS (1993) Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. Nature 366:740–742
Lu P, Takai K, Weaver VM, Werb Z (2011) Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol 3:pii: a005058
Lyons TJ, Basu A (2012) Biomarkers in diabetes: hemoglobin A1c, vascular and tissue markers. Transl Res 159:303–312
Lyons TJ, Bailie KE, Dyer DG, Dunn JA, Baynes JW (1991) Decrease in skin collagen glycation with improved glycemic control in patients with insulin-dependent diabetes mellitus. J Clin Invest 87:1910–1915
MacLeod AS, Havran W (2011) Functions of skin resident γδ T cells. Cell Mol Life Sci 68:2399–2408
Mascai E, Takats Z, Derzbach L, Korner A, Vasarhelyli (2013) Verification of skin autofluorescence values by mass spectrometry in adolescents with type 1 diabetes: brief report. Diabetes Technol Ther 15:1–4
Madison KC (2003) Barrier function of the skin: "La raison d'etre" of the epidermis. J Invest Dermatol 121:231–241
Marschall S, Sander B, Mogensen M, Jørgensen TM, Andersen PE (2011) Optical coherence tomography—current technology and applications in clinical and biomedical research. Anal Bioanal Chem 400:2699–2720
Maynard JD, Rohrscheib M, Way JF, Nguyen CM, Ediger MN (2007) Non-invasive type 2 diabetes screening. Superior sensitivity to fasting plasma glucose and A1C. Diabetes Care 30:1120–1124
Mayrovitz HN, McClymont A, Pandya N (2013) Skin tissue water assessed via tissue dielectric constant measurements in persons with and without diabetes mellitus. Diabetes Technol Ther 15:1–6
McAleer MA, Irvine AD (2013) The multifunctional role of filaggrin in allergic skin disease. J Allergy Clin Immunol 131:280–291
Millioni R, Puricelli L, Iori E, Trevisan R, Tessari P (2012) Skin fibroblasts as a tool for identifying the risk of nephropathy in the type 1 diabetic population. Diabetes Metab Res Rev 28:62–70
Miura T, Suzuki W, Ishihara E, Arai I, Ishida H, Seino Y, Tanigawa K (2001) Impairment of insulin-stimulated GLUT4 translocation in skeletal muscle and adipose tissue in the Tsumura Suzuki obese diabetic mouse: a new genetic animal model of type 2 diabetes. Eur J Endocrinol 145:785–790
Miyata S, Yamada N, Kawada T (2012) Possible involvement of hypothalamic nucleobindin-2 in hyperphagic feeding in Tsumura-Suzuki obese diabetes mice. Biol Pharmacol Bull 35:1784–1793
Monnier VM, Bautista O, Kenny D, Sell DR, Fogarty J, Dahms W, Cleary PA, Lachin J, Genuth S (1999) Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes. Diabetes 48:870–880
Monnier VM, Mustata GT, Biemel KL, Reihl O, Lederer MO, Zhenyu D, Sell DR (2005) Cross-linking of the extracellular matrix by the Maillard reaction in aging and diabetes. An update on "a puzzle nearing resolution". Ann N Y Acad Sci 1043:533–544
Monnier VM, Sell DR, Dai Z, Nemet I, Collard F, Zhang J (2008) The role of the Amadori product in the complications of diabetes. Ann N Y Acad Sci 1126:81–88
Monnier VM, Sell DR, Strauch C, Sun W, Lachin JM, Cleary PA, Genuth S (2013) The association between skin collagen glucosepane and past progression of microvascular and neuropathic complications in type 1 diabetes. J Diabetes Complications 27:141–149
Moore JM, Frew IDO (1965) Peripheral vascular lesion in diabetes mellitus. BMJ 2:19–23
Mulder DJ, Water T, Lutgers HL, Graaff R, Gans RO, Zijlistra F, Smit AJ (2006) Skin autofluorescence, a novel marker for glycemic and oxidative stress-derived advanced glycation endproducts: an overview of current clinical studies, evidence, and limitations. Diabetes Technol Ther 8:523–535
Muller LMAJ, Gorter KJ, Goudzwaard WL, Schellevis FG, Hoepelma IM (2005) Increased risk of common infections in patients with type1 and type2 diabetes mellitus. Clin Infect Dis 41:281–288
Nestle FO, Di Meglio P, Qin J-Z, Nickoloff B (2009) Skin immune sentinels in health and disease. Nat Rev Immunol 9:679–691
Ngo BT, Hayes KD, DiMiao DJ, Srinivasan SK, Huerter CJ, Rendell MS (2005) Manifestations of cutaneous diabetic microangiopathy. Am J Clin Dermatol 6:225–237

Noordzij MJ, Mulder DJ, Oomen PHN, Brouwer T, Jager J, Castro Cabezas M, Lefrand JD, Smit AJ (2012) Skin autofluorescence and risks of micro- and macrovascular complications in patients with type 2 diabetes mellitus—a multicentre study. Diabet Med 29:1556–1561

O'Shea M, Teeling M, Bennett K (2013) The prevalence and ingredient cost of chronic comorbidity in the Irish elderly population with medication treated type 2 diabetes: a retrospective cross sectional study using a national pharmacy claims database. BMC Health Serv Res 13:23

Pageon H (2010) Reaction of glycation and human skin: the effects on the skin and its components, reconstructed skin as model. Pathol Biol 58:226–231

Paliwal S, Hwang BH, Tsai KY, Mitragotri S (2013) Diagnostic opportunities based on skin biomarkers. Eur J Pharm Sci 50:546-556

Park HY, Kim HJ, Jung M, Chung CH, Hasham R, Park CS, Choi EH (2011) A long-standing hyperglycaemic condition impairs skin barrier by accelerating skin ageing process. Exp Dermatol 20: 969–974

Paul RG, Bailey AJ (1996) Glycation of collagen: the basis of its central role in the late complications of ageing and diabetes. Int J Biochem Cell Biol 28:1297–1310

Pleitez MA, Lieblein T, Bauer A, Hertzberg O, von Lilienfeld-Toal H, Mäntele W (2013) In vivo noninvasive monitoring of glucose concentration in human epidermis by mid-infrared pulsed photoaucstic spectroscopy. Anal Chem 15:1013–1020

Proksch E, Brandner JM, Jensen J-M (2008) The skin: an indispensable barrier. Exp Dermatol 17:1063–1072

Quondamatteo F (2002) Assembly, stability and integrity of basement membranes in vivo. Histochem J 34:369–381

Ragunatha S, Anitha B, Inamadar AC, Palit A, Devarmani SS (2011) Cutaneous disorders in 500 diabetic patients attending diabetic clinic. Indian J Dermatol 56:160–164

Redel H, Gao Z, Aleksyenko AV, Zhou Y, Perez-Perez GI, Weinstock G, Sodegren E, Blaser MJ (2013) Quantitation and composition of cutaneous microbiota in diabetic and non-diabetic men. J Infect Dis 207:1105–1114

Rees DA, Alcolado JC (2005) Animal models of diabetes mellitus. Diabet Med 22:359–370

Rendell M, Saxena S, Shah D (2003) Cutaneous blood flow and peripheral resistance in type II diabetes compared to intermittent claudication patients. Int J Angiol 12:166–171

Rodgers KE, Ellefson DD, Espinosa T, Hsu Y, Di Zerega G, Mehiran-Shai R (2006) Expression of intracellular filament, collagen, and collagenase genes in diabetic and normal skin after injury. Wound Repair Regen 14:298–305

Romani N, Brunner PM, Stingl G (2012) Changing views of the role of Langerhans cells. J Invest Dermatol 132:872–881

Sakai S, Endo Y, Ozawa N, Sugawara T, Kusaka A, Sayo T, Tagami H, Inoue S (2003) Characteristics of the epidermis and stratum corneum of hairless mice with experimentally induced diabetes mellitus. J Invest Dermatol 120:79–85

Sakai S, Kikuchi K, Satoh J, Tagami H, Inoue S (2005) Functional properties of stratum corneum in patients with diabetes mellitus: similarities to senile xerosis. Br J Dermatol 153:319–323

Sangiorgi S, Manelli A, Reguzzoni M, Ronga M, Protasoni M, Dell'Orbo C (2010) The cutaneous microvascular architecture of human diabetic toe studied by corrosion casting and scanning electron microscopy analysis. Anat Rec 293:1639–1645

Sasaki T, Larsson H, Tisi D, Claesson-Welsh L, Hohenester E, Timpl R (2000) Endostatins derived from collagens XV and XVIII differ in structural and binding properties, tissue distribution and anti-angionenic activity. J Mol Biol 01:1179–1190

Schaefer C, Biermann T, Schroeder M, Fuhrhop I, Niemeier A, Ruther W, Algenstaedt P, Hansen-Algenstaedt N (2010) Early microvascular complications of prediabetes in mice with impaired glucose tolerance in dyslipidemia. Acta Diabetol 47:S19–S27

Schmidt AM, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, Esposito C, Hegarty H, Hurley W, Clauss M, Wang F, Pan Y-CE, Tsang TC, Stern D (1992) Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. J Biol Chem 267:14987–14997

Schramm JC, Dinh T, Veves A (2006) Microvascular changes in the diabetic foot. Int J Low Extrem Wounds 5:149–159

Seirafi H, Farsinejad K, Firooz A, Davoudi SM, Robati RM, Hoseini MS, Ehasani AH, Sadr B (2009) Biophysical characteristics of skin in diabetes: a controlled study. J Eur Acad Dermatol Venereol 23:146–149

Sell DR, Biemel KM, Reihl O, Lederer MO, Strauch CM, Monnier VM (2005) Glucosepane is a major protein cross-link of the senescent human extracellular matrix. J Biol Chem 280:12310–12315

Shah BR, Hux JE (2003) Quantifying the risk of infectious diseases for people with diabetes. Diabetes Care 26:510–513

Simpson CL, Patel DM, Green KJ (2011) Deconstructing the skin: cytoarchitectural determinants of epidermal morphogenesis. Nat Rev Mol Cell Biol 12:565–580

Sivanandam S, Anburajan M, Venkartraman B, Menaka M, Sharath D (2012) Medical thermography: a diagnostic approach for type diabetes based on non-contact infrared thermal imaging. Endocrine 42: 343–351

Stirban A, Nandrean S, Negrean M, Koschinsky T, Tschoepe D (2008) Skin autofluorescence increases postprandially in human subjects. Diabetes Technol Ther 10:200–205

Suaya JA, Eisenberg DF, Fang C, Miller LG (2013) Skin and soft tissue infections and associated complications among commercially insured patients aged 0–64 years with and without diabetes in the US. PLOS ONE 8:e60057

Sugawara T, Iwamoto N, Akashi M, Kojima T, Hisatsune J, Sugai M, Furuse M (2013) Tight junction dysfunction in the stratum granulosum leads to aberrant stratum corneum barrier function in claudin-1 deficient mice. J Dermatol Sci 70:12–18

Suzuki W, Iizuka S, Tabuchi M, Funo S, Yanagisava T, Kimura M, Sato T, Endo T, Kawamura H (1999) A new mouse of spontaneous diabetes derived from ddY strain. Exp Anim 48:181–189

Tahrani AA, Zeng W, Sakher J, Piya MK, Hughes S, Dubb K, Stevens MJ (2012) Cutaneous structural and biochemical correlates of foot complications in high-risk diabetes. Diabetes Care 35:1913–1918

Takahashi N, Takasu S (2011) A close relationship between type 1 diabetes and vitamin A deficiency and matrix metalloproteinase and hyaluronidase activities in skin tissues. Exp Dermatol 20:899–904

Tanaka K, Tani Y, Asai J, Nemoto F, Kusano Y, Suzuki H, Hayshi Y, Asahi K, Nakayama M, Miyata T, Watanabe T (2012) Skin autofluorescence is associated with severity of vascular complications in Japanese patients with type 2 diabetes. Diabet Med 29:492–500

Taylor KR, Costanzo AE, Jameson JM (2011) Dysfunctional γδ T cells contribute to impaired keratinocyte homeostasis in mouse models of obesity. J Invest Dermatol 131:2409–2418

Tellechea A, Kafanas A, Leal EC, Tecilazich F, Kuchibhotla S, Auster ME, Kontoes I, Paolino J, Carvalho E, Nabzdyk LP, Veves A (2013) Increased skin inflammation and blood vessel density in human and experimental diabetes. Int J Low Extrem Wounds 12:4–11

Tessier FJ (2010) The Maillard reaction in the human body. The main discoveries and factors that affect glycation. Pathol Biol 58:214–219

Thyssen JP, Linneberg A, Carlsen BC, Johansen JD, Engkilde K, Hansen T, Pociot F, Pedersen O, Meldgaard M, Scecsi PB, Stender S, Menne T (2011) A possible association between a dysfunctional skin barrier (filaggrin null-mutation status) and diabetes: a cross-sectional study. BMJ Open 1:e000062

Van Agtmael T, Bruckner-Tuderman L (2010) Basement membranes and human disease. Cell Tissue Res 339:167–188

Van der Pijl JW, Daha MR, Born J, Verhagen NAM, Lemkes HHPJ, Bucala R, Berden JHM, Zwinderman AH, Bruijn JA, van Es LA, van der Woude F (1998) Extracellular matrix in human diabetic nephropathy: reduced expression of heparan sulphate in skin basement membrane. Diabetologia 41:791–798

Van Hattem S, Bootsma AH, Thio BT (2008) Skin manifestations of diabetes. Cleve Clin J Med 75:772–787

Vashist SK (2012) Non-invasive glucose monitoring technology in diabetes management: a review. Anal Chim Acta 750:16–27

Vasioukhin V, Bauer C, Degenstein L, Wise B, Fuchs E (2001) Hyperproliferation and defects in epithelial polarity upon conditional ablation of α-catenin in skin. Cell 104:605–617

Von Mering J, Minkowski O (1890) Diabetes mellitus nach Pankreasexstirpation. Arch Exp Pathol Pharmakol 26:371–387

Wanke I, Skabytska Y, Kraft B, Peschel A, Bidermann T, Schittek B (2013) *Staphylococcus aureus* skin colonization is promoted by barrier disruption and leads to local inflammation. Exp Dermatol 22:153–155

Wells-Knecht MC, Lyons TJ, McCance DR, Thorpe SR, Baynes JW (1997) Age-dependent increase in ortho-thyrosine and methionine sulfoxide in human skin collagen is not accelerated in diabetes. J Clin Invest 100:839–846

Wohlrab J, Wohlrab D, Meiss F (2007) Skin diseases in diabetes mellitus. J Dtsch Dermatol Ges 5:37–53

Ye X, Cheng X, Liu L, Zhao D, Dong Y (2013) Blood glucose fluctuation affects skin collagen metabolism in the diabetic mouse by inhibiting the mitogen-activated protein kinase and Smad pathways. Clin Exp Dermatol 38:530–538

Zakharov P, Talary MS, Kolm I, Caduff A (2010) Full-field optical coherence tomography for the rapid estimation of epidermal thickness: study of patients with diabetes mellitus type 1. Physiol Meas 31:193–205

Zafar H, Enfield J, O'Connell M-L, Ramsay B, Lynch M, Leahy MJ (2013) Assessment of psoriatic plaque in vivo with correlation mapping optical coherence tomography. Skin Res Technol. doi:10.1111/srt.12096

Zam A, Dsouza R, Hrebesh M, Subhash HM, Marie-Louise O'Connell M-L, Enfield J, Larin K, Leahy MJ (2013) Feasibility of correlation mapping optical coherence tomography (cmOCT) for anti-spoof sub-surface fingerprinting. J Biophotonics 6:663–667

Zhao G, Usui M, Underwood RA, Singh PK, James GA, Stewart PS, Fleckman P, Olerud JE (2012) Time course study of delayed wound healing in a biofilm-challenged diabetic mouse model. Wound Repair Regen 20:342–352

Zhu P, Ren M, Yang C, Hu YX, Ran J, Li Y (2011) Involment of RAGE, MAPK and NF-κB pathways in AGEs-induced MMP-9 activation in HaCaT keratinocytes. Exp Dermatol 21:123–129

Zimmet P, Alberti KGMM, Shaw J (2001) Global and societal implications of the diabetes epidemic. Nature 414:782–787