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UNDERSTANDING PREMATURE AND NEWBORN SKIN ANATOMY

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Human skin is exquisite in design. As the only fully self-renewing organ, it is a vigilant, protective, early detection, and sensing surface derived from the same tissue as the brain. The development (fabrication, construction) of this exquisite structure occurs and gains functionality *in utero* over many months. Premature infants are born before the development is complete. Remarkably, although it is immature, premature neonatal skin is designed to carry on its development in extraordinarily different conditions of the dry, cool, nonaqueous environment at birth. To optimize outcomes, it is essential to understand and thereby facilitate this transition. In this context, full-term (FT) skin is an important comparator, along with well-studied adult skin. Currently, the limit of human viability is ~22 weeks and the requisite skin care is exceptionally challenging.

Newborn infants depend upon a robust innate immune system, provided by the stratum corneum (SC) and epidermis, physical, antimicrobial protection, and adaptation to the dry, cool, nonsterile environment at birth. Neonatal skin provides essential functions, including: 1) immune-surveillance and infection control; 2) barrier to water loss (inside) and irritants (outside); 3) acid mantle formation, *i.e.*, provision of an acidic skin pH; 4) resilience of mechanical trauma; 5) tactile discrimination; and 6) thermal regulation.

This chapter first describes skin development throughout gestation, encompassing fetal, premature, and FT stages. It next examines the postnatal development, namely the biological process that is prompted by the transition from an aqueous uterine environment to the relatively dry gaseous surroundings at birth. This adaptation/maturation varies based on gestational age (GA) at birth, particularly for infants born before 37 weeks of gestation. The purpose is to discuss the current knowledge of strategies to facilitate skin development and innate immunity and the information gaps that present challenges in the care of the most fragile of infants.

Neonatal skin development

Fetal skin

A single layer of ectoderm generates one layer of epithelial cells, the basal layer, that contains keratinocyte cells (Figure 1.1A). Basal layer fetal keratinocytes prompt nearby fetal fibroblasts to make type VII collagen, type IV collagen and laminin A to form the dermal-epidermal basement membrane beneath the keratinocytes (Figure 1.1B).[1] The structural proteins of this basement membrane create stability and

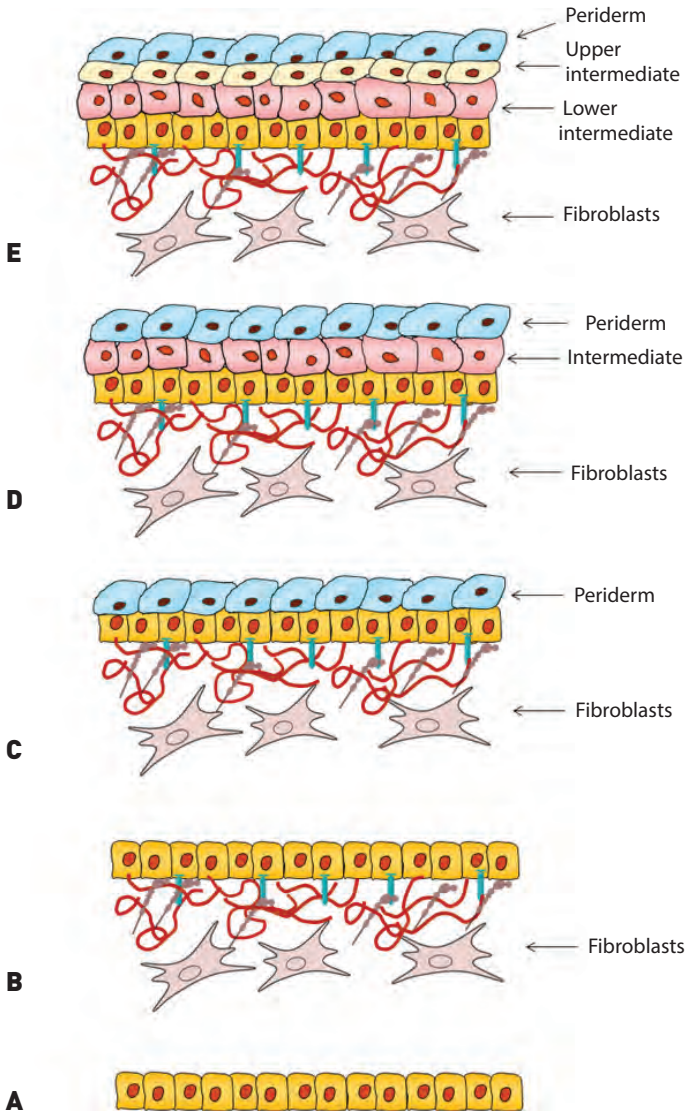

FIGURE 1.1. Progression of epidermal differentiation over time. The process of epidermal formation beginning with a single layer from ectoderm. A) The ectoderm generates one layer of epithelial cells, the basal layer, that contains keratinocyte cells. B) Basal layer fetal keratinocytes prompt nearby fetal fibroblasts to make type VII collagen, type IV collagen, and laminin A to form the dermal-epidermal basement membrane beneath the keratinocytes. C) At 7-9 weeks, the basal layer stratifies to produce the keratin-17 containing periderm, a layer that can also proliferate. D) Over time in the periderm, keratin-17 decreases, keratin-6 increases and further stratification forms a third layer, the intermediate layer between the basal layer and periderm. E) At weeks 14-23, four layers were noted, with upper and lower intermediate layers.

attach the structures together. The keratinocyte cells reside in this layer where they proliferate until they detach (delaminate) and begin to move upwards.[2] The basal layer also contains stem cells and attaches melanocytes and Merkel cells.[3-5] Specialized structured, desmosomes and adherens junctions, interconnected the basal layer cells.[6] At 7-9 weeks, the basal layer stratifies to produce the keratin-17 containing periderm, a layer that can also proliferate Figure 1.1C).[6] Over time in the periderm, keratin-17 decreases, keratin-6 increases and further stratification forms a third layer, the intermediate layer between the basal layer and periderm (Figure 1.1D). The periderm continues to proliferate with fetal growth. Structurally, fetal periderm contains tight junctions that hold adjacent cells together to prevent migration of smaller species from above and below.[7] The protein claudin-6 is present in periderm tight junctions, but not in epidermal tight junctions.[8] Keratin family proteins appear during epidermal development, beginning with keratin-8 and keratin-18 in the initial single cell layer.[9] Proliferation in the basal cells gives rise to keratin-5 and keratin-14, prior to further stratification.[10] Keratin-14 is present in the basal layer starting at weeks 13-14.[11] Keratin-10 is in the intermediate and periderm layers at weeks 13-14 and only in intermediate cells at week 16.[4, 10, 11]

Further stratification

Over time, proteins that ultimately form the cornified envelope appear, including transglutaminase (TG1-3), involucrin (INV), loricrin (LOR), small proline-rich proteins (SPRP), lamellar granule associated proteins (LGP), cystatin A, and elafin (PI3).[12, 13] At gestational weeks 7-9, the periderm contains TG1-3, INV, SPRP2/3, and LGP; although TG1-3 are faintly present in the basal layer (Table 1.I).[12] In the three-layer epidermis of weeks 9-23, TG1-3 was in all layers but LOR and SPRP2/3 were only in the periderm. INV was in the periderm and intermediate layers. At weeks 14-23, four layers were noted, with upper and lower intermediate layers (Figure 1.1E). TG1-3 was in each layer, although weakly in the basal layer. Around 23 weeks, five layers were noted with the periderm disappearing in some areas. TG1-3 were throughout, LGP was in both intermediate layers and the cornified layer and INV, SPRP2/3 and LOR in the upper intermediate and cornified layer. Proteins from the fetal skin samples were discerned as three layers, namely periderm (called the granular layer), intermediate (labeled as the spinous layer) and the basal layer (Table 1.II).[14] INV was in the periderm and intermediate layers at gestational weeks 14, 16, 20, 22, 24, and 32. LOR was found in periderm only at 16, 20, 22, 24, and 32 weeks. SPRP1 in periderm at 16, 20, 22, 24, and 32 weeks and in intermediate at 24 and 32 weeks. Filaggrin appeared in the periderm only at 24 and 32 weeks. The order of appearance parallels that of mature skin cornified envelope. These that proteins are forming in the aqueous uterine environment.

The cornified cell envelope forms as the periderm regresses.[12] Figure 1.2 shows a ~23-week fetus with periderm, keratinized squames, and hair on the skin surface from a scanning electron micrograph. At ~26 weeks, the epidermis is keratinized with no evidence of periderm, 2-3 epidermal (spinous) layers and 5-6 SC layers of SC.[15] The cornification of the cell envelope as periderm regresses may be one aspect of protection of the epidermis from amniotic fluid.[12] The appearance of these proteins (*i.e.*, INV, LOR, SPRP, TGase, filaggrin) by week ~24 is like their appearance in mature, homeostatic adult skin.[2, 14] Said differently, the periderm regression process is analogous to cornification in developed skin. Presently neonates as young as 21-22 weeks are considered viable. Consequently, the features of their skin structure that were first published decades ago are essential to understand. Does the cornification at this stage in development protect against hydration effects of the amniotic fluid and, perhaps, the high humidity environments of periviable infants nursed in neonatal intensive care units?

TABLE 1.I. Timeline of structural proteins in developing skin.[12]

Age/time	Layers	Description	Proteins
49-65 days	Two	Periderm	TG1-3, involucrin, SPRP2/3, LGP
7-9 weeks		Basal	TG1-3 (weak)
66-160 days	Three	Periderm	TG1-3, involucrin, loricrin, SPRP2/3, LGP
9-23 weeks		Intermediate	TG1-3, involucrin, LGP
		Basal	TG1-3
96-160 days	Four*	Periderm	TG1-3, involucrin, loricrin, SPRP2/3, LGP
14-23 weeks		Upper intermediate	TG1-3, involucrin
		Lower intermediate	TG1-3
		Basal	TG1-3 (weak)
>160 days	Four or more	Periderm	TG1-3 not present when periderm regressed, LGP
>23 weeks		Interfollicular keratinization	TG1-3
		Upper intermediate (granular)	TG1-3, LGP
		Lower intermediate (spinous)	TG1-3, LGP
		Basal	TG3 increased
>160 days	Four or more	Keratinized epidermis, membrane 15 nm	TG1-3 throughout
>23 weeks			Involucrin, SPRP2/3, loricrin in granular and cornified
			Loricrin weak in cornified layer
			LGP in spinous, granular, cornified

LGP: lamellar granule associated proteins; SPRP: small proline-rich proteins; TG: transglutaminase.
*Cell membrane was 15 nm, comparable to adult cornified envelope.

Hair follicles

Hair follicles begin to form in the basal layer around week 12.[16] Cells of the follicle infundibulum and hair canal terminally differentiate and cornify at week 14. Recent studies at week 14, hair follicle cells included cuticle, cortex, inner root sheath, outer root sheet companion layer, matrix, and placode.[17] Terminal differentiation and cornification take place between hair follicles takes place around week 24. Keratinization occurs in follicular scalp skin at 17-19 weeks of GA and later in follicular abdominal skin at 22 weeks of GA.[18] Interfollicular keratinization occurs at 20-21 weeks of GA and 23-24 weeks of GA for head and abdominal skin, respectively. Consider a figure for follicular then interfollicular keratinization. The interfollicular regions have cornified throughout by about 24 weeks of GA and remnants of periderm may be visible.[16] The total time for development of keratinized epidermal cells is 16 weeks, from weeks 8 to 24.[16] Eccrine glands commence development at week 13 and finish around week 24.[3] The hair bulge, upper infundibulum, hair germ, isthmus, junctional region, and sebaceous gland all contain

TABLE 1.II. Timeline of structural proteins in later gestation.[14]

Age	Layers	Proteins
14	Periderm	Involucrin
	Intermediate	Involucrin
	Basal	
16	Periderm	Involucrin, loricrin, SPRR1
	Intermediate	Involucrin
	Basal	
20	Periderm	Involucrin, loricrin, SPRR1
	Intermediate	Involucrin
	Basal	
22	Periderm	Involucrin, loricrin, SPRR1
	Intermediate	Involucrin
	Basal	
24	Periderm	Involucrin, loricrin, SPRR1, filaggrin
	Intermediate	Involucrin, SPRR1
	Basal	
32	Periderm	Involucrin, loricrin, SPRR1, filaggrin
	Intermediate	Involucrin, SPRR1
	Basal	

SPRR: small proline-rich protein.

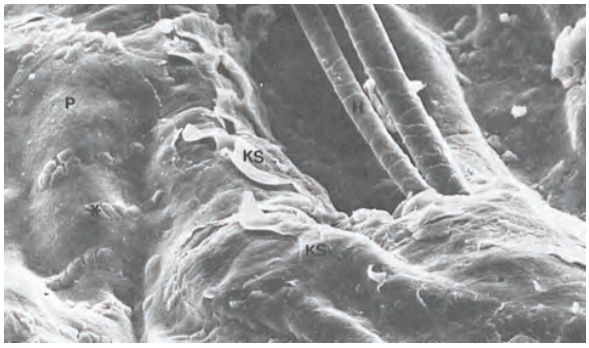

FIGURE 1.2. Skin surface micrograph of a ~23-week fetal skin surface: a ~23-week fetal skin surface with periderm, keratinized squames, and hair on the skin surface from a scanning electron micrograph.

stem cells.[19] Single cell transcriptomic analyses of interfollicular epidermal cells (neonatal foreskin) identified four distinct basal stem cell types.[20] Two were at the rete ridges (top, bottom) and two resided between the basal and suprabasal layers, but attached to the basement membrane.

Dendritic cells

Dendritic innate immune cells, Langerhans cells (LCs) appear by gestational week 6.[21] Their proliferation is triggered by keratinocytes to increase in density and form a network in the skin by week 12,[22] Merkel cells are in the basal and first observed at gestational week 8.[4, 23] They respond to mechanical stimuli, *e.g.*, touch, by releasing norepinephrine that activates action potentials at the synapse during transduction.[24, 25] Meissner corpuscles are low threshold mechanoreceptors of touch, pressure and vibration located in the dermis just below the epidermis. They begin to develop at weeks 22-24 but do not achieve adult-like structures until postnatal month 8.[25, 26]

Dermis

Over gestational weeks 7 to 26, the dermis thickens and becomes more organized as the fibrous extracellular matrix (ECM) increases.[27] By week 12, bundles of fibrous structures have formed in varying orientations relative to the epidermis.[28] Two dermal layers, *i.e.*, reticular, and papillary, have formed by gestational week 26. At birth, FT neonatal dermal fiber bundle thickness, size and composition is "between" fetal and adult tissue.[28] The fetal reticular dermis at week 24 has some fine elastic fibers that become more abundant within the reticular and papillary layers by weeks 29-30. Compared to adults, fetal and neonatal dermis is less structured and the cell differentiation rate is higher.[29] Animal

studies suggest that low collagen levels occur until postnatal day 10-15.[30] After birth, connective tissue increases.[31] Gene set enrichment analysis found ECM organization and collagen fibril organization to be in the top 15 significantly enriched themes in full thickness neonatal skin samples compared to ultraviolet (UV) exposure protected adult skin.[32]

Vasculature

The cutaneous vasculature system develops in the dermis and parallel to the epidermis by weeks 6-7 with capillary structures and vessel wall structures visible with transmission electron microscopy.[33] Two vessel planes occur around weeks 7-11 and vessel density is higher in older specimens. Dynamic microcirculation was evaluated in FT infants (N.30, mean GA weeks 39.7±1.2, day of life 2-3) and premature infants (N.20, mean GA weeks 27.7±2.5, weekly until discharge) at the inner upper arm using a MicroScan video microscope in the Sidestream Dark Field imaging method.[34] After accounting for oxygen saturation, heart rate, and arterial blood flow, premature infants had a higher percent of small diameter vessels (68.3±14.1), a lower percent of medium diameter vessels (30.4±13.) and a lower percent of large diameter vessels (1.3±0.9) than FT infants, where values were 48.5±9.9, 42.2±7.2, and 6.3±4.2, respectively. For premature infants, the differences remained at weeks 2-5, but the functional vessel density (mm/mm^2) decreased with increasing postnatal age.

Skin thickness

Skin thickness at thigh, deltoid, and suprascapular sites ranged from 0.87 to 1.87 mm in 70 FT and premature (≥34 weeks of GA) infants measured with an 18-MHz B mode ultrasound probe.[35] The skin was thicker in FT than in premature infants. The suprascapular site was thicker than the other sites in premature infants. A recent meta-analysis of fetal and neonatal skin thickness by histology reported high variability and, therefore, inaccuracy in skin thickness measurements.[36] The forearm skin thickness was 1390.8±873 μm in 217 infants from 24 to 42 weeks of GA, measured with a 20 MHz ultrasound probe.[37] The forearm epidermal thickness was 174.6±17.5 μm and the corresponding dermal thickness was 1244.5±869, for a dermal to epidermal ratio of 6.8.

Vernix caseosa

Of particular interest are the features of the tissue and adnexa that are associated with the capability of the production of vernix caseosa, produced during the last trimester of gestation. Vernix appears at the fetal eyebrows as early as 17-19 weeks of gestation, progressing from head to toe and back to front, covering the entire surface over time.[38] Corticotropic-releasing factors from the placenta and/or hypothalamus are believed to stimulate the release adrenocorticotropic hormone by the pituitary gland, in turn prompting release of androgenic steroids by the adrenal gland.[39] They become active androgens and function within the sebaceous gland. Vernix contains ~80% water, 10.3% protein, and 9.7% lipids.[40, 41] The water is associated with anuclear cells, likely from the hair follicles and/or from the infundibular section of sebaceous glands.[42, 43] The flattened cells are coated with a nonlamellar mixture of nonpolar and polar lipids, including triglycerides, wax esters and squalene from sebaceous gland[43, 44] and cholesterol, fatty acids (FAs) and ceramides that are believed to derive from the epidermis.[43, 45] Intercellular desmosomes were not evident and the extent of keratinization suggested relative immaturity, *i.e.*, from fetal skin.[41, 46] These findings suggest that vernix "extrudes" through the hair shaft, onto the skin surface at the hair and over the entire interfollicular epidermis throughout gestation.[42] The vernix covering is hydrophobic and

protects the epidermis from water and amniotic fluid, thereby creating a "drier" condition for cornification, *i.e.*, SC formation.[47]

Vernix from the forehead of neonates of 30-41 weeks of GA had higher levels of cholesterol while infants closer to term birth had higher squalene levels.[48] The squalene increase may indicate greater sebaceous gland activity just before birth. The FA composition of vernix differed by GA. Monounsaturated and branched-chain FAs were higher in FT infants whereas mono-saturated and polyunsaturated FAs were lower compared to premature infants (29-36 weeks).[49] The sphingomyelin fraction of sphingolipids was lower at younger gestations.[50] Vernix from FT infants includes proteins that are known for innate immune functions, including ubiquitin, psoriasin (S100A7), lysozyme (LYZ), secretory leukocyte protease inhibitors (SLPIs) and protease/enzyme inhibitors calgranulin A (S100A8) and calgranulin B (S100A9).[51] Vernix reduced the activity of group B *Streptococcus*, *Klebsiella pneumoniae* and *Listeria monocytogenes*, known perinatal pathogens.[52]

The hydrophobic vernix coating protects the developing SC from water, thereby allowing epidermal development via cornification to occur.[47] It impedes penetration of potential irritants, *e.g.*, enzymes, in the amniotic fluid and maintained native enzyme activity needed for epidermal development.[53] When the fetal lungs are mature, they secrete phospholipid surfactants that, in turn, detach some vernix from the skin surface.[54] The amniotic fluid becomes cloudy. The fetus swallows the vernix-containing fluid, presumably to prepare the intestine for extra-utero nutrition. Retention of vernix on the skin of FT infants increased skin hydration, decreased skin surface acidity and reduced skin erythema at birth.[38] Infants born at <29 weeks of gestation have limited, if any, vernix exposure, raising questions as to the impact on skin maturation after birth.

Full-term infant skin structure and postnatal adaptation

Skin barrier and TEWL

Healthy FT infants have a functional epidermal barrier at birth. Figure 1.3 shows the multilayered epidermis, with the basement membrane, basal, spinous (lower intermediate), granular (upper intermediate), and SC, representing FT gestation. The dermis contains the vasculature and various structural proteins. Transepidermal water loss (TEWL), a measure of epidermal barrier integrity, is low with values of ~4-8 g/m^2/h, comparable to normal adult values.[55-58] Optimum functioning of human skin depends upon having sufficient hydration for tissue flexibility/movement and for desquamation of the outer SC layers during homeostasis.[59] The degree of skin hydration depends upon the environmental conditions, the presence of vernix, and body site.[38, 60] FT neonatal skin hydration decreases rapidly over postnatal day 1, increases over 14 days, further increasing over the first month.[61] Visible dryness/scaling appear, due, in part, to the low levels of water binding amino acids and small molecules, collectively known as natural moisturizing factor (NMF).[62, 63] Filaggrin (filament aggregating protein) binds to intermediate keratin filaments, structures that couple to corneodesmosomes that, in turn, join corneocytes together, within and between SC layers. Filaggrin undergoes proteolysis to produce NMF. This process is delayed in the high moisture *in utero* but upregulated with exposure to drier conditions at birth.[64] NMF production increases after birth, along with skin hydration (Figure 1.4).[62]

Skin surface acidity, pH

The skin surface acidity, measured as apparent pH, is around seven at birth, decreases rapidly over postnatal days 1-4 and continues to decrease over at least the next three months as the acid mantle develops.[65]

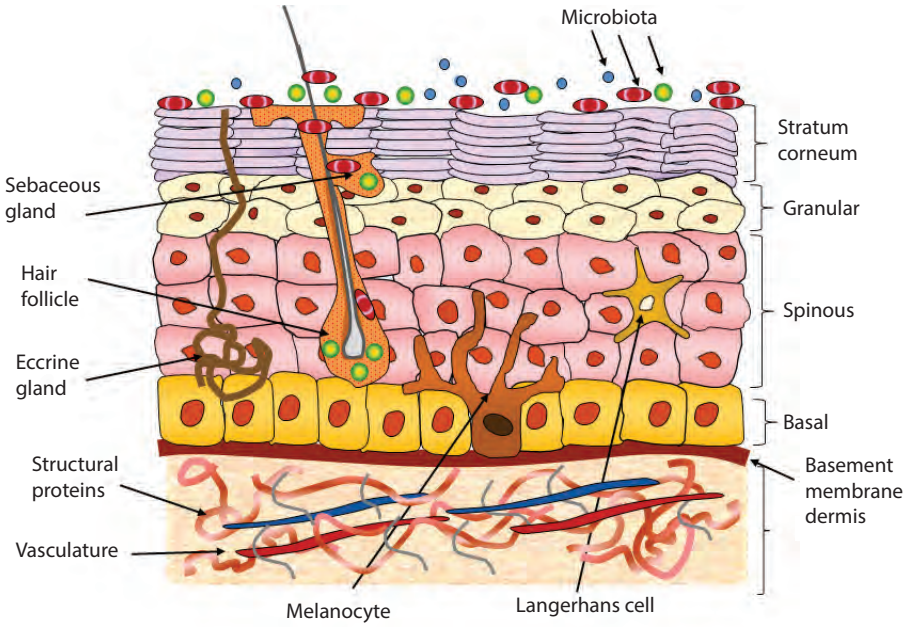

FIGURE 1.3. Skin structure at full-term. The multilayered epidermis, with the basement membrane, basal, spinous (lower intermediate), granular (upper intermediate), and SC, representing full-term gestation. The dermis contains the vasculature and various structural proteins. Transepidermal water.
SC: stratum corneum.

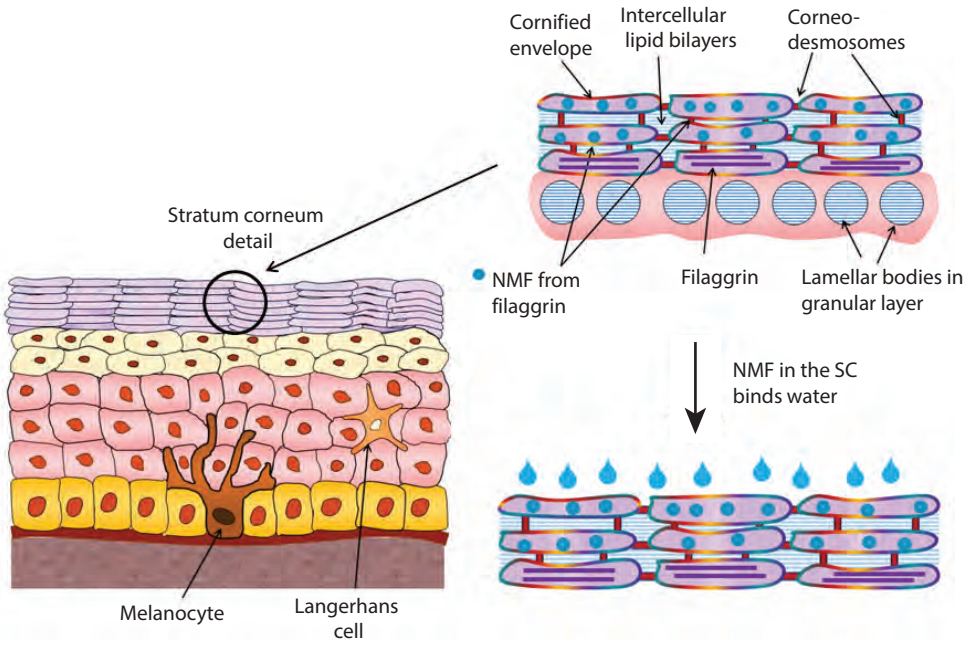

FIGURE 1.4. Skin surface hydration. How the stratum corneum generates water binding materials. Filaggrin (filament aggregating protein) binds to intermediate keratin filaments, structures that couple to corneodesmosomes that, in turn, join corneocytes together, within and between stratum corneum layers. Filaggrin undergoes proteolysis to produce NMF. This process is delayed in the high moisture *in utero* but upregulated with exposure to drier conditions at birth. NMF production increases after birth, along with skin hydration.
NMF: natural moisturizing factor; SC: stratum corneum.