Cyanoacrylate Skin Surface/ Follicular Stripping

22

Gérald E. Piérard, Claudine Piérard-Franchimont, Philippe Delvenne, and Pascale Quatresooz

22.1 Introduction

The stratum corneum (SC) is a dead structure. However, it exerts a unique barrier function partly protecting the living tissues from a series of environmental threats including ultraviolet light, microorganisms and irritant/toxic xenobiotics. In addition, the SC controls any excessive loss in water, electrolytes and macromolecules from the skin. In addition, the SC acts as a unique sophisticated biosensor that signals the underlying epidermis to respond to various external stimuli. Despite minimal metabolic activity, the SC corresponds to a highly specialised structure resulting from the continuous corneocyte renewal ideally keeping a steady state in the SC structure and thickness. However, corneocytes are structurally and biochemically heterogeneous.

Over a vast part of the body, the SC is typically composed of 12–16 layers of flattened corneocytes. These cells are about 1 μm thick and have a mean area reaching approximately 1,000 μm^2. Of note, the corneocyte surface area is influenced by age, anatomical location and any conditions including chemical irritation and UV insults altering the epidermal renewal. In particular, the average corneocyte size apparently increases with age. This feature is assumed to be related to a prolonged transit time of corneocytes through the SC.

Normal SC binds water and keeps its surface soft and smooth. Some of its molecular components bind water and/or prevent water evaporating from the skin surface. These compounds include the so-called natural moisturising factor (NMF), consisting of a mixture of water-soluble small molecules such as amino acids, lactate and urea, the intercellular lipids, sebum and specific protein components of corneocytes. Abnormalities in these components produce a harsh and hard SC that leads to the development of fine cracking and fissuring.

In some instances, the SC homeostasis is altered. Indeed, the SC is the repository of many biologic events that previously influenced the underlying metabolically active keratinocytes. The SC structure is further altered by diverse and repeat external insults. The genetic background, the nutritional status, some physical agents, as well as drugs, cosmetics, toiletries and other chemical xenobiotics represent additional modulators of the SC structure. Knowledge about the fine SC structure is crucial in many respects in the field of dermocosmetic science, particularly when dealing with age-related xerosis and effects of surfactants, emollients and squamolytic agents.

G.E. Piérard, MD, PhD (✉)
C. Piérard-Franchimont • P. Delvenne • P. Quatresooz
Laboratory of Skin Bioengineering and Imaging,
Department of Dermatopathology,
University Hospital of Liège, CHU,
Sart Tilman, Liège B-4000, Belgium
e-mail: gerald.pierard@ulg.ac.be

E. Berardesca et al. (eds.), *Non Invasive Diagnostic Techniques in Clinical Dermatology*,
DOI 10.1007/978-3-642-32109-2_22, © Springer Berlin Heidelberg 2014

22.2 Cyanoacrylate Skin Surface and Follicular Stripping

Cyanoacrylate skin surface stripping (CSSS) is a time-honoured method [1]. The method came into existence when high bond glues became available. After its initial description, it was soon applied for diagnostic purposes [2]. Sampling on polyethylene strips was a decisive improvement in its subsequent development [3]. Indeed, such plastic sheet is preferable to glass slide for two main reasons. It is indeed easier to get a close modelling of curved body areas. In addition, the adhesion of the SC is such that corneocytes are not lost during the laboratory procedures.

The CSSS method consists of depositing a drop of cyanoacrylate liquid adhesive onto a supple transparent sheet of terephthalate polyethylene, 175 μm thick, cut to the size of a conventional coverslip (1.5×6 cm). The sampling material is presently commercially available as a kit (S-Biokit, C+K electronic, Cologne, Germany). The material is pressed firmly onto the target site of the skin. After 15–30 s, a sheet of SC is easily harvested. Because the adhesion relies on a chemical reaction, the depth of harvested SC is determined by the depth of penetration of the adhesive before polymerisation. The cleavage level is exclusively located inside the SC [4, 5].

CSSS can be performed on any part of the body, with two main provisos. On the one hand, sampling from a hairy area is painful because of pulling out hairs, and the CSSS quality is inadequate owing to the erratic contact with the SC. It is therefore advisable to shave these areas before any CSSS harvesting. On the other hand, the natural intercorneocyte cohesion on the palms and soles is usually stronger than the cyanoacrylate bond, thus impairing the collection of a uniform sheet of corneocytes. However, a CSSS sampling on these sites is possible in certain physiopathologic conditions in which the SC texture is compromised. Of note, oozing and eroded lesions cannot be assessed by CSSS.

When vellus hairs are present on the examined site, they are captured with the CSSS. In addition, CSSS collects follicular casts corresponding to the horny material present at the opening of the pilosebaceous follicles at the skin surface. This sampling method has been specifically called follicular biopsy [6]. It is therefore possible to assess the density of the follicles per unit of surface area and to observe the presence of follicular hyperkeratosis (ketosis), as well as comedones, *Trichostasis spinulosa*, intrafollicular bacteria and mites [4–16]. The so-called skin pores corresponding to follicular or sudoral openings at the skin surface are possibly explored using CSSS [17].

22.3 Global Aspect of Normal Skin on CSSS

CSSS of normal skin reveals a regular network of high-peaked crests corresponding to the skin surface microdepressions composed of the primary, secondary and tertiary order lines [1–5, 18]. Their patterns are typical for specific parts of the body. The primary lines of the skin surface correspond to grooves in the latticework papillary relief at the dermoepidermal junction [19–21]. In young individuals, intersections of primary and secondary lines delimit regularly shaped polyhedral plateaus. On stretching of the skin surface a realignment of these lines occurs. With aging, this network progressively loses its configuration, aligning itself preferentially along the skin tension lines and ending by disappearing in the shallow wrinkles [4]. It is therefore possible to indirectly assess the texture of the superficial dermis on CSSS. As a result, dermal aging, corticosteroid-induced atrophy, sclerosis, striae distensae, scars and many other changes in the connective tissue are conveniently observed noninvasively using CSSS. Such morphologic assessment of the skin microrelief is possibly quantified by computerised image analysis using any profilometry method [4, 5].

Cytologic characteristics of corneocytes are hardly visible on CSSS unless histologic dyes are used [4, 5, 22]. A number of stains are suitable. The most useful and simplest one is a mixture of toluidine blue and basic fuchsin in 30 % ethanol [4]. Each corneocyte contains a water-insoluble protein complex made predominantly of a highly

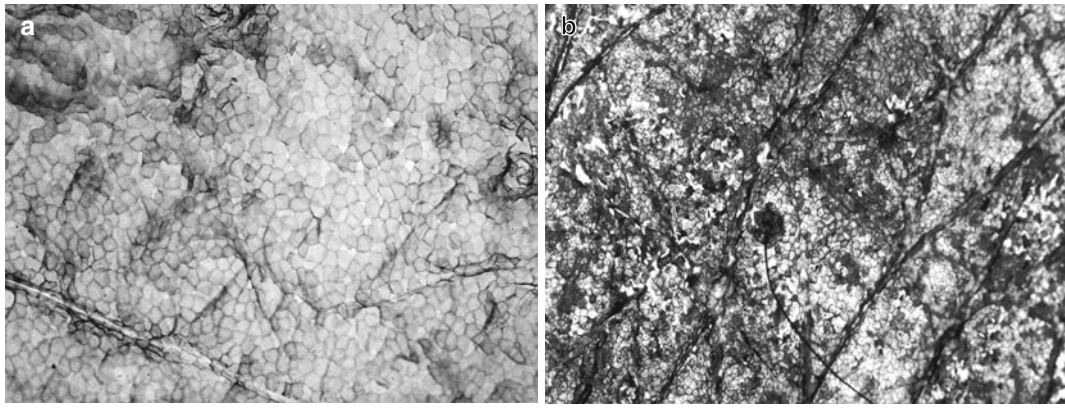

Fig. 22.1 Corneosurfametry bioassay. (**a**) Normal control. The limits of some corneocytes are visible. (**b**) Harsh surfactant. The fragile immature corneocytes are heavily stained in an irregular pattern

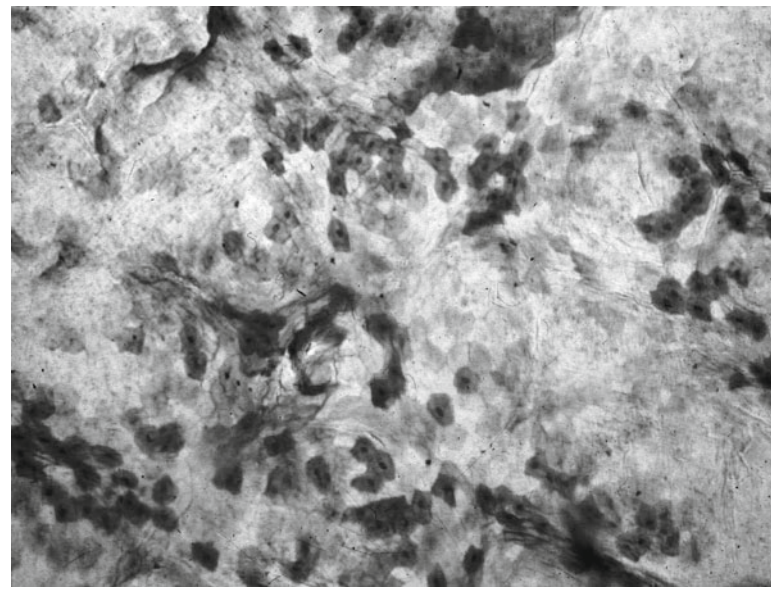

Fig. 22.2 Parakeratotic cells dispersed in the stratum corneum

organised keratin microfibrillar matrix. Such a structure is encapsulated in a protein and lipid-enriched shell. The cornified cell envelope shows differences in maturation among corneocytes. Basically, two distinct types of cornified cell envelopes are distinguished, namely, the fragile immature envelopes and the rigid mature ones [23, 24]. The former cells are recognised on CSSS used in the corneosurfametry bioassay (Fig. 22.1).

Normal skin exhibits a regular cohesive pattern of adjacent anucleated corneocytes. Their boundaries are clearly stained by a thin polyhedral rim. Parakeratotic cells are usually rare and they are not clustered on healthy skin (Fig. 22.2). They are recognised by the presence of a nucleus central to the polyhedral cell.

Saprophytic microorganisms are normally confined to the skin surface and the appendages. Thus, they are encased in the cyanoacrylate bond during sampling and they are not accessible to the staining procedure. As a result, the surface microflora is not seen on CSSS. By contrast, microorganisms present in the follicular casts can be collected distinctly from the skin surface

microflora by scraping out the horny spiky structures appending to the CSSS. Viability of the intrafollicular bacteria is possibly assessed using flow cytometry [25, 26].

Sensitive skin is a condition of reduced cutaneous tolerance to environmental factors (cold, heat, wind, wool topical products, etc.). Clinical manifestations consist mainly of subjective symptoms and sensory irritation including discomfort, itching, stinging and burning sensations. No specific signs are discernable on regular CSSS.

22.4 Diagnostic CSSS in Inflammatory Dermatoses

The SC exhibits both biologic efficiency and aesthetic qualities, but it is rarely considered as a structure causing serious disablement when diseased. However, some 50 % of the clinical workload of dermatologists deals with disorders causing scaling and/or thickening of the SC. Psoriasis, the ichthyotic disorders, the various eczematous diseases and the xerosis panel are such disturbances. These relatively common conditions are characterised by abnormal epidermal maturation and scaling. Despite their frequency in most populations, and the problems they cause, there has been relatively little research on the clinical consequences of the abnormal cornification. CSSS help exploring these disorders. Obviously, the diagnostic indications for CSSS are limited to disorders characterised by SC involvement. The most common conditions that can be diagnosed by CSSS are summarised in Table 22.1 [2–5, 27–33].

Definite diagnoses are reached in superficial infectious and parasitic skin diseases [2–5, 27, 29, 31]. Morphologic examination, possibly combined with fungal cultures, can be carried out to identify these types of diseases. By essence, infectious agents that are made visible on CSSS are not those adhering to the skin surface (see above), but rather those invading the SC. Fungi, including yeasts and dermatophytes, show their typical morphology, forming clusters or network of globular or filamentous structures.

Table 22.1 Indications for surface biopsy

1. Superficial infections
Molluscum contagiosum
Bacterial diseases (impetigo, erythrasma, etc.)
Dermatophytosis
Candidosis
Pityriasis versicolor
2. Superficial parasitoses
Scabies
Demodicidosis
Oxyuriasis
3. Xeroses and erythemato-squamous, spongiotic and parakeratotic dermatitides
Xerosis, ketosis and ichthyosis
Eczema – contact dermatitis
Atopic dermatitis
Pityriasis rosea
Id reaction
Psoriasis
Seborrheic dermatitis
4. Tumours
Malignant melanoma
Melanocytic nevus
Dysplastic nevus
Seborrheic keratosis

In the group of parasitic disorders, scabies may pose a problem at the time of sampling [2, 5]. In fact, this diagnosis can be established only when the mite, its eggs or its dejecta are present in the sample. Duplicate or a series of CSSS samplings should therefore be taken from suspected scabies burrows. The first one intends to remove the roof of the burrow. The following ones have a better chance to collect the parasite. Any samples taken outside such parasitic lesion, for example, from non-specific prurigo, will be unhelpful because the diagnosis will merely suggest the presence of a spongiotic dermatitis [4, 5, 31]. Demodex mites are conveniently recognised [4, 5, 15] and highlighted in the follicular casts by the Fite stain [4].

Non-infectious erythemato-squamous disorders include spongiotic and parakeratotic dermatoses and xeroses [4, 5, 29, 31]. Spongiotic dermatoses represent superficial inflammatory reactions responsible for spongiosis, microvesiculation and

serosity leakage inside the SC. Contact dermatitis, atopic dermatitis and pityriasis rosea are examples that belong to this group of diseases [5]. Parakeratotic dermatoses encompass id reactions, chronic eczema and stable psoriasis. Seborrheic dermatitis comes within this category, particularly when *Malassezia* yeasts are rare. In active psoriasis, clusters of neutrophils are found on top of parakeratotic foci.

22.5 Diagnostic CSSS in Cutaneous Neoplasms

Some epithelial neoplasms display characteristic aspects on CSSS [4, 5]. Seborrheic keratoses show spotty lenticular foci of soft hyperkeratosis. Widening of shallow furrows with hyperkeratosis is present. Samples of actinic keratosis often exhibit irregular thickness with interfollicular parakeratosis and xerosis. Actinic porokeratosis is revealed by the rim of cornoid lamellation and loss of the normal microrelief inside the lesion. Verrucous surfaces overlying melanocytic nevi and dermatofibromas are less pathognomonic, but sharp circumscription by a normal-looking surrounding skin and uniformity of the changes in the texture of the SC are usually seen in a benign neoplasm.

In CSSS taken from pigmented neoplasms, melanin can be found inside corneocytes or in atypical melanocytes. Melanin located only inside corneocytes is a feature of benign neoplasms, such as lentigines and solar lentigines. Presence of atypical melanocytes in the SC is strongly suggestive of malignant melanoma, but also, in rare instances, of a benign melanoacanthoma [4, 5, 34–36]. Thus, CSSS proves to be sensitive and specific in the distinction between malignant melanoma and benign melanocytic tumours such as common melanocytic nevi, dysplastic nevi or pigmented seborrheic keratoses [27]. For research purposes, karyometry of neoplastic melanocytes can be performed on CSSS [35, 36]. Basal cell carcinomas and squamous cell carcinomas do not exhibit specific or suggestive features on CSSS.

22.6 CSSS Analytic Measurements

22.6.1 Xerosis Grading

Some aspects of disease severity and/or improvement are conveniently assessed noninvasively on CSSS showing specific features in the SC. An example is given by xeroses which correspond to various forms of predominantly orthokeratotic hyperkeratosis [37]. Such SC structure corresponds to the so-called dry skin, although the condition recalls some aspects of the ichthyoses [4, 5, 31, 37–39]. Several grades of orthokeratotic hyperkeratosis are detected on CSSS [5]. Type 0 refers to the absence of hyperkeratosis, except for some discrete focal accumulation of corneocytes in the primary lines of the skin. Type 1a corresponds to a continuous linear hyperkeratosis of the primary lines. Type 1b is characterised by hyperkeratosis predominant at the site of adnexal openings either at hair follicles or at acrosyringia. Type 2 corresponds to focal hyperkeratosis of the skin surface plateaus representing less than 30 % of the surface of the sampling. Type 3 resembles type 2 but with an altered area over 30 % of the skin CSSS. Type 4 is defined by a homogeneous and diffuse hyperkeratosis with persistence of the primary lines. Type 5a resembles type 4 but with loss of recognisable primary lines. Type 5b corresponds to the most heterogeneous and diffuse hyperkeratosis with loss or marked remodelling of the primary line network.

22.6.2 Corneofungimetry

Some quantifications of disease severity and therapeutic activity can be performed on CSSS using computerised image analysis. Quantifications of the fungal load in dermatomycosis can be performed similarly to what described in the corneofungimetry bioassay [30–33].

22.6.3 Corneomelametry

Melanin is present in corneocytes of normal skin in phototype V and VI individuals. The dusty melanin

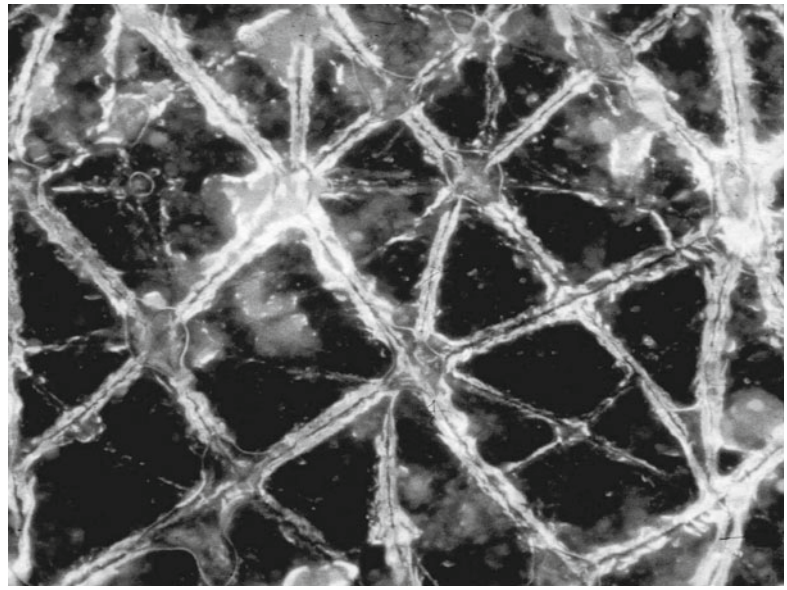

Fig. 22.3 Fluorescent dye persistence in a stratum corneum renewal test

load is specifically revealed using argentaffin-staining procedures. The relative darkness of these CSSS can be assessed using corneomelametry [40–42]. This method consists of measuring the reduction of light transmission through the CSSS using a photometric device designed for photomicroscopy. It is important to distinguish melanin-laden anucleated corneocytes from neoplastic dendritic melanocytes having migrated inside SC overlying a malignant melanoma.

22.6.4 Stratum Corneum Dynamics

The dynamics of SC renewal is conveniently assessed using CSSS collected about 10 days after topical application of a fluorescent or a coloured dye. The more the SC renewal is rapid, the less stain remains present on the CSSS.

Dansyl chloride is a time-honoured fluorescent compound for the SC. For years, the test relied on daily assessment of the decline in the clinical fluorescence [43]. The rate of SC renewal was determined by the duration of the fluorescence persistence. However, this clinical test proved to be difficult to interpret because it was not easy to clinically evaluate with precision the moment of fluorescent loss. This was due to the uneven fade-out of fluorescence persistence. The CSSS method is a variant performed at a fixed time after dansyl chloride application. The fluorescence pattern is quantified using image analysis [44]. The shallow skin lines represent a typical site for residual fluorescence (Fig. 22.3).

Fluorescence fading is assessed in vivo after application of topical products and interpreted as an effect on the keratinocytes proliferation [45]. However, this procedure represents a pitfall when the test product extracts dansyl chloride from the SC [46]. The correct procedure should begin with the application of the test product for a dozen of days. In a second step dansyl chloride should be applied without any further applications of the test product. Such a procedure allows to disclose any boosting effect on the epidermis without any risk for artefactual dansyl chloride extraction.

A risk of allergy and systemic resorption of dansyl chloride is possible. Hence, there is some limitation for its use, particularly in subjects involved in a series of similar tests. Dihydroxyacetone was offered as a surrogate SC marker [47].

22.6.5 Comedometry

Comedometry allows the computerised quantification of the number and size of follicular casts present on CSSS (Fig. 22.4). This method

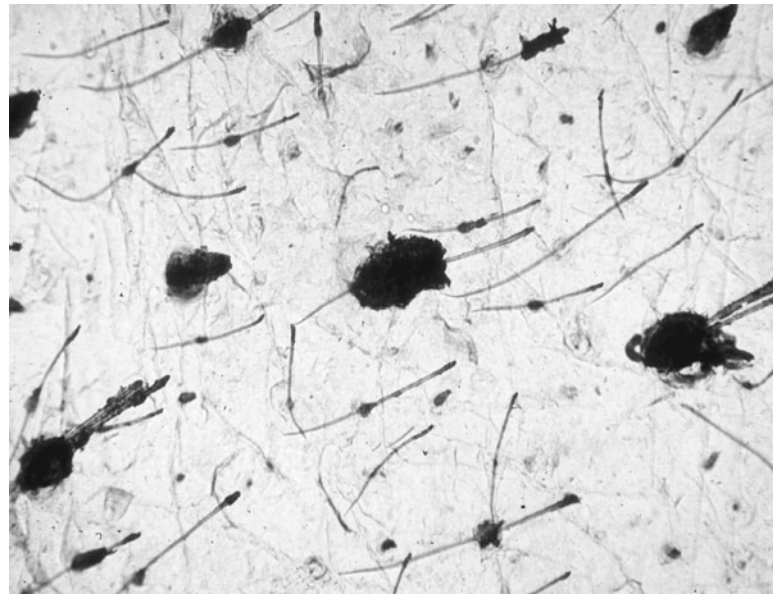

Fig. 22.4 Comedometry showing a few microcomedones (follicular casts) dispersed in an otherwise normal stratum corneum

finds application in the comedogenesis and comedolysis-related disorders and treatments [9, 10, 14, 25, 26]. Comedometry on human skin appears more relevant than animal (rabbit ear) models of comedogenesis. There are large interindividual differences in the number of horny follicular casts among subjects. When an exogenous comedogenic factor is involved, the vast majority of the follicles are similarly affected. By contrast, endogenous comedogenic factors typically affect at variable degree a minority of follicles. The sensitivity of the method is such that microcomedolysis is possibly objectivated after a few days or weeks of adequate treatment.

Lipid-sensitive foils are conveniently used to assess the sebum output at the skin surface. It is possible to combine this method with CSSS [48]. In a first step, the foil is applied to the skin for a limited period of time not exceeding 1 h. The outlines of the foil are ink marked on the SC. In a second step following removal of the foil, a CSSS is collected from the very same skin site. The ink mark is visible on this sampling. The CSSS and the foil are then exactly superposed using the ink mark as an adjusting mark. The dual samplings are examined under the microscope and submitted to image analysis considering the darker horny follicular casts and the clear transparent sebum spots. Correlations are possibly established between the follicular pore sizes, microcomedones and the follicular sebum output [48].

Conclusions

Beside conventional biopsies and cytology of exudates, imprints and scrapings, CSSS provide useful information in the field of dermatopathology and skin pharmacology. This simple and noninvasive method allows the clinician to avoid invasive biopsy within limits of well-defined indications. Less than 3 min are necessary between sampling and examination. There are evident features and subtle characteristics discernible in the structure of the SC that enable a diagnosis to be made in a variety of skin diseases. It is important to stress that no single criterion should usually be relied upon for a definitive diagnosis on CSSS. Rather a constellation of clues should be sought. Quantifications are made possible on CSSS using computer-assisted image analysis.

Acknowledgements This work was supported by a grant from the "Fonds d'Investissement de la Recherche Scientifique" of the University Hospital of Liège. No other

sources of funding were used to assist in the preparation of this manuscript. The authors have no conflicts of interest that are directly relevant to the content of this review. The authors appreciate the excellent secretarial assistance of Mrs. Ida Leclercq and Marie Pugliese.

References

1. Marks R, Dawber RPR (1971) Skin surface biopsy: an improved technique for the examination of the horny layer. Br J Dermatol 84:117–123
2. Agache P, Mairey J, Boyer JP (1972) Cyanoacrylate stratum corneum stripping. Interest in skin physiology and pathology. J Med Lyon 53:1017–1022
3. Lachapelle JM, Gouverneur JC, Boulet M, Tennstedt D (1977) A modified technique (using polyester tape) of skin surface biopsy. Br J Dermatol 97:49–52
4. Piérard-Franchimont C, Piérard GE (1987) Assessment of aging and actinic damages by cyanoacrylate skin surface stripping. Am J Dermatopathol 9:500–509
5. Piérard-Franchimont C, Piérard GE (1985) Skin surface stripping in diagnosing and monitoring inflammatory, xerotic and neoplastic diseases. Pediatr Dermatol 2:180–184
6. Mills OH, Kligman AM (1983) The follicular biopsy. Dermatologica 167:57–63
7. Holmes RL, Williams M, Cunliffe WJ (1972) Pilosebaceous duct obstruction and acne. Br J Dermatol 87:327–332
8. Marks R, Dawber RPR (1972) In situ microbiology of the stratum corneum. Arch Dermatol 105:216–221
9. Mills OH, Kligman AM (1982) A human model for assaying comedolytic substances. Br J Dermatol 107:543–548
10. Mills OH, Kligman AM (1982) A human model for assessing comedogenic substances. Arch Dermatol 118:903–905
11. Groh DG, Mills OH, Kligman AM (1992) Quantitative assessment of cyanoacrylate follicular biopsies by image analysis. J Soc Cosmet Chem 43:101–112
12. Pagnoni A, Kligman AM, El Gammal S, Stoudemayer T (1994) Determination of density of follicles on various regions of the face by cyanoacrylate biopsy: correlation with sebum output. Br J Dermatol 131: 862–865
13. Piérard GE, Piérard-Franchimont C, Goffin V (1995) Digital image analysis of microcomedones. Dermatology 190:99–103
14. Letawe C, Boone M, Piérard GE (1998) Digital image analysis of the effect of topically applied linoleic acid on acne microcomedones. Clin Exp Dermatol 23:56–58
15. Forton F, Seys B, Marchal JL, Song AM (1998) Demodex folliculorum and topical treatment: acaricidal action evaluated by standardized skin surface biopsy. Br J Dermatol 138:461–466
16. Uhoda E, Piérard-Franchimont C, Piérard GE (2003) Comedolysis by a lipohydroxyacid formulation in acne prone subjects. Eur J Dermatol 13:65–68
17. Uhoda E, Piérard-Franchimont C, Petit L, Piérard GE (2005) The conundrum of skin pores in dermocosmetology. Dermatology 210:3–7
18. Hashimoto K (1974) New methods for surface ultrastructure: comparative studies of scanning electron microscopy, transmission electron microscopy and replica method. Int J Dermatol 13:357–381
19. Piérard GE, Hermanns JF, Lapière CM (1974) Stereology of the dermo-epidermal interface. Scanning electron microscopy observation of the basement membrane plasticity. Dermatologica 149:266–273
20. Piérard GE, Franchimont C, Lapière CM (1980) Aging, its expression in the skin microanatomy. Int J Cosmet Sci 2:209–214
21. Rochefort A, Makki S, Agache P (1986) Anatomical location of human skin furrows. Clin Exp Dermatol 11:445–449
22. Marks R (1972) Histochemical applications of skin surface biopsy. Br J Dermatol 86:20–26
23. Hirao T, Denda M, Takahashi M (2001) Identification of immature cornified envelopes in the barrier-impaired epidermis by characterization of their hydrophobicity and antigenicities of the components. Exp Dermatol 10:35–44
24. Harding CR, Long S, Richardson J, Rogers J, Zhang Z, Bush A, Rawlings AV (2003) The cornified cell envelope: an important marker of stratum corneum maturation in healthy and dry skin. Int J Cosmet Sci 25:157–167
25. Piérard-Franchimont C, Gaspard V, Lacante P, Rhoa M, Slachmuylders P, Piérard GE (2000) A quantitative biometrological assessment of acne and hormonal evaluation in young women using a triphasic low-dose oral contraceptive containing gestodene. Eur J Contracept Reprod Health Care 5:275–286
26. Piérard-Franchimont C, Goffin V, Arrese JE, Martalo C, Braham C, Slachmuylders P, Piérard GE (2002) Lymecycline and minocycline in inflammatory acne a randomized, double-blind study on clinical and in vivo antibacterial efficacy. Skin Pharmacol Appl Skin Physiol 15:112–119
27. Whiting DA, Bisset EA (1974) The investigation of superficial fungal infections by skin surface biopsy. Br J Dermatol 91:57–65
28. Knudsen EA (1975) The areal extent of dermatophyte infection. Br J Dermatol 92:413–416
29. Piérard GE, Piérard-Franchimont C, Dowlati A (1992) Skin surface biopsy in clinical and experimental dermatology. Rev Eur Dermatol MST 4:445–466
30. Piérard GE, Piérard-Franchimont C, Arrese EJ (1993) Comparative study of the activity and lingering effect of topical antifungals. Skin Pharmacol 6:208–214
31. Piérard-Franchimont C, Piérard GE (1995) Surface biopsies and skin diseases. Rev Med Liege 50:7–15
32. Arrese JE, Fogouang L, Piérard-Franchimont C, Piérard GE (2002) Euclidean and fractal computer-

assisted corneofungimetry. A comparison of 2% ketoconazole and 1% terbinafine topical formulations. Dermatology 204:222–227
33. Piérard GE, Piérard-Franchimont C, Quatresooz P (2010) Updating corneofungimetry: a bioassay exploring dermatomycoses and antifungal susceptibility. Mycopathologia 169:27–35
34. Piérard GE, Piérard-Franchimont C, Arrese EJ et al (1989) Cyanoacrylate skin surface strippings as an improved approach for distinguishing dysplastic nevi from malignant melanomas. J Cutan Pathol 16: 180–182
35. Piérard-Franchimont C, Piérard GE (1989) The value of morphometry and of surface biopsy in the detection of malignant melanoma. Rev Med Liege 44: 610–614
36. Piérard GE, Ezzine Sebai N, Fazaa B et al (1995) Karyometry of malignant melanoma cells present in skin strippings. Skin Res Technol 1:177–179
37. Piérard-Franchimont C, Piérard GE (1984) Xeroses: structure of harsh skin. Int J Cosmet Sci 6:47–54
38. Piérard GE (1996) EEMCO guidance for the assessment of dry skin (xerosis) and ichthyosis: evaluation by stratum corneum strippings. Skin Res Technol 2:3–11
39. Piérard-Franchimont C, Piérard GE (2002) Beyond a glimpse at seasonal dry skin. A review. Exog Dermatol 1:3–6
40. Hermanns JF, Petit L, Piérard-Franchimont C, Paquet P, Piérard GE (2002) Assessment of topical hypopigmenting agents on solar lentigines of Asian women. Dermatology 204:281–286
41. Petit L, Piérard GE (2003) Analytic quantification of skin lightening by a 2% hydroquinone-cyclodextrin formulation. J Am Acad Dermatol Venereol 17: 546–549
42. Thirion L, Piérard-Franchimont C, Piérard GE (2006) Whitening effect of a dermocosmetic formulation. A randomized double-blind controlled study on melasma. Int J Cosmet Sci 28:263–267
43. Janssen LH, Hojko-Tomoko MT, Kligman AM (1974) Improved fluorescence staining technique for estimating turnover of the human stratum corneum. Br J Dermatol 90:9–12
44. Piérard GE (1992) Microscopic evaluation of dansyl chloride test. Dermatology 185:37–40
45. Ridge BD, Batt MD, Palmer HE, Jarett A (1988) The dansyl chloride technique for stratum corneum renewal as an indicator of changes in epidermal mitotic activity following topical treatment. Br J Dermatol 118:167–174
46. Paye M, Smion A, Piérard GE (1994) Dansyl chloride labelling of stratum corneum: its rapid extraction from skin can predict skin irritation due to surfactants and cleansing products. Contact Dermatitis 30:91–96
47. Piérard GE, Piérard-Franchimont C (1993) Dihydroxyacetone test as a substitute for the dansyl chloride test. Dermatology 186:133–137
48. Piérard GE (1987) Rate and topography of follicular sebum excretion. Dermatologica 175:280–283