International Journal of Cosmetic Science, 2007, **29**, 241–275

Review Article

Nail biology and nail science

D. A. R. de Berker*, J. André† and R. Baran‡

*Bristol Dermatology Centre, Bristol Royal Infirmary, Bristol BS2 8HW, U.K., †Department of Dermatology, CHU Saint Pierre, 1000 Brussels, Belgium and ‡Nail Disease Centre, 42, rue des Serbes, 06400 Cannes, France

Received 7 January 2007, Accepted 25 January 2007

Keywords: electron microscopy, imaging of the nail apparatus, immunohistochemistry, nail apparatus, nail unit

Synopsis

The nail plate is the permanent product of the nail matrix. Its normal appearance and growth depend on the integrity of several components: the surrounding tissues or perionychium and the bony phalanx that are contributing to the nail apparatus or nail unit. The nail is inserted proximally in an invagination practically parallel to the upper surface of the skin and laterally in the lateral nail grooves. This pocket-like invagination has a roof, the proximal nail fold and a floor, the matrix from which the nail is derived. The germinal matrix forms the bulk of the nail plate. The proximal element forms the superficial third of the nail whereas the distal element provides its inferior two-thirds. The ventral surface of the proximal nail fold adheres closely to the nail for a short distance and forms a gradually desquamating tissue, the cuticle, made of the stratum corneum of both the dorsal and the ventral side of the proximal nail fold. The cuticle seals and therefore protects the ungual cul-de-sac. The nail plate is bordered by the proximal nail fold which is continuous with the similarly structured lateral nail fold on each side. The nail bed extends from the lunula to the hyponychium. It presents with parallel longitudinal rete ridges. This area, by contrast to the matrix has a firm attachment to the nail plate and nail avulsion produces a denudation of the nail bed. Colourless, but translucent, the highly vascular connective tissue containing glomus organs transmits a pink colour through the nail. Among its multiple functions, the nail provides counterpressure to the pulp that is essential to the tactile sensation involving the fingers and to the prevention of the hypertrophy of the distal wall tissue, produced after nail loss of the great toe nail.

Correspondence: Robert Baran, Nail Disease Centre, 42, rue des Serbes, 06400 Cannes, France.
Tel.: + 33 4 93 39 99 66; fax: + 33 4 92 98 80 30; e-mail: baran.r@club-internet.fr

Résumé

L'appareil unguéal repose directement sur le périoste de la phalange distale. Il comprend quatre structures épithéliales spécialisées: la matrice, qui produit l'ongle, le lit sur lequel il repose, le repli sus-unguéal qui le cache en partie et l'hyponychium dont il se détache L'ongle (tablette ou lame cornée) est une plaque de kératine faite de plusieurs couches de cellules cornées, dont la production s'effectue de façon continue tout au long de la vie. De forme presque rectangulaire, semi-transparent, il doit sa couleur rose aux vaisseaux qui parcourent le lit unguéal sous-jacent. Il adhère fortement à son lit, mais de façon réduite à la matrice et sur ses bords latéraux. Le repli sus-unguéal (proximal ou postérieur) recouvre environ le 1/5èmepostérieur de la tablette unguéale à la surface de laquelle il adhère fortement. Il se termine par une production cornée, la cuticule qui scelle l'espace virtuel du cul-de-sac unguéal où s'enfonce la racine de la lame selon un angle aigu, pratiquement parallèle à la surface cutanée. Ce repli sus-unguéal possède une face dorsale anatomiquement identique à la peau du doigt. Les 3/4

Olaplex EX2003
Wella v. Olaplex
PGR2026-00010

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

antérieurs de sa face ventrale constituent l'éponychium dont la couche cornée participe à la formation de la cuticule, tandis que la matrice proximale tapisse son quart postéro-inférieur. La forme générale de la matrice rappelle celle d'un croissant, à concavité postéro-inférieure, ses cornes latérales s'abaissent davantage aux orteils qu'aux doigts. La maturation et la différenciation des kératinocytes de la matrice aboutissent à la formation de l'ongle. Le tiers supérieur de la tablette unguéale provient de la matrice proximale; les 2/3 inférieurs sont issus de la matrice distale et des cellules du lit. La lunule, seule partie visible de la matrice, n'est fortement développée qu'aux pouces. Elle apparaît sous forme d'une zone semi-lunaire blanc opaque à convexité distale. Elle détermine la direction et la forme générale du bord libre de l'ongle. Le caractère lâche du derme lunulaire a été mis en lumière à la faveur d'études couplant IRM et histologie. Le *lit* de l'ongle s'étend de la lunule à l'hyponychium. La kératinisation de l'épithélium s'effectue en l'absence de couche granuleuse et produit l'ongle ventral. L'architecture dermo-épidermique du lit montre un arrangement fait de crêtes et de sillons longitudinaux où courent les capillaires. L'hyponychium possède un épithélium identique à celui de la sole dont la kératinisation s'effectue par l'intermédiaire d'une couche granuleuse. La limite distale de l'hyponychium est marquée par un sillon à convexité antérieure. Il forme un espace sous-unguéal où s'accumulent les cellules de la couche cornée. L'oblitération de cette région est réalisée au cours du ptérygion ventral. Dans certaines dermatoses, comme le psoriasis, l'hyperkératose sous-unguéale distale semble traduire un réveil du pouvoir onychogène de l'hyponychium. Dans les régions proximale et latérales, l'ongle est serti dans les rainures correspondantes, bordées par les replis latéraux, en continuation avec le repli proximal. Ces replis fournissent une voie anatomique continue pour la propagation des processus pathologiques. La profondeur des rainures latérales augmente à mesure qu'elles atteignent la rainure proximale avec laquelle elles se confondent. L'intégrité anatomo-physiologique de l'appareil unguéal repose à la fois sur les structures épidermiques précédentes et sur l'os qui sert d'axe à la phalangette, et dont il épouse la convexité. Parmi les multiples fonctions de l'ongle, la protection du lit prend toute sa valeur en traumatologie, sa couverture évite une kératinisation responsable d'un faux-ongle du lit. Mais avant tout, la tablette constitue un plan de contre-pression, essentiel à la sensibilité tactile pulpaire et à la prévention de l'hypertrophie des tissus mous distaux, fréquente après l'avulsion unguéale du gros orteil.

Introduction

The nail is the permanent product of the nail matrix. Its normal appearance and growth depend on the integrity of several components: the surrounding tissues and the bony phalanx that are contributing to the nail apparatus.

We have tried to extend the term 'Nail biology' by 'Nail science' to highlight the multiple facets of this useful appendage.

The physical properties of the nail and the different types of imaging of the nail apparatus supplement this article.

Gross anatomy and terminology

The nail is a window to the nail bed held in place by the nail folds, its origin at the matrix and attachment to the nail bed. It ends at a free edge distally [1–3]. At its proximal margin it lies adjacent to the distal interphalangeal joint. The insertion of the extensor tendon of this joint lies 1.2 mm from the matrix which has bearing on the common biological and surgical process that affect these two structures [4]. Serial dissections of 19 fingers suggest that the surface markers of the boundaries of the matrix lie at 75% of the distance from the cuticle to the crease of the distal interphalangeal joint and laterally to the sagittal midline of the digit [5]. Anatomical terms are illustrated in Figs 1 and 2. The definitions of the components of the nail unit are as follows:

Nail plate (nail): Durable keratinized structure which continues growing throughout life.

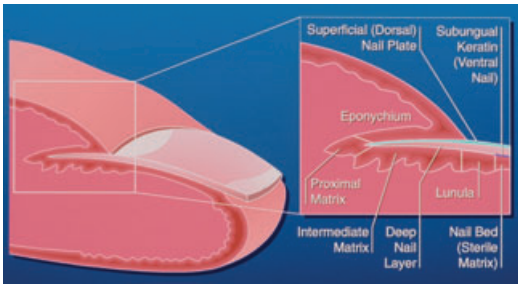


Figure 1 Anatomy of the nail apparatus.

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

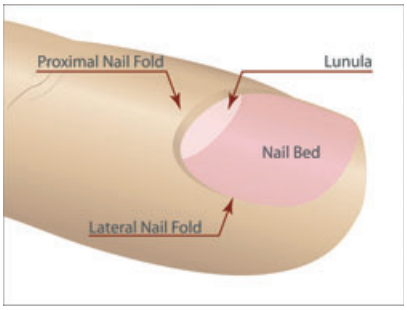


Figure 2 The tip of a digit showing the component parts of the nail apparatus.

Lateral nail folds: The cutaneous folded structures providing the lateral borders to the nail.
Proximal nail fold (posterior nail fold): Cutaneous folded structure providing the visible proximal border of the nail, continuous with the cuticle. On the lower portion of the undersurface, this becomes the dorsal matrix.
Eponychium: Refers to the upper portion of the ventral aspect of the proximal of the proximal nail fold and adheres closely to the nail for a short distance.
Cuticle: The stratum corneum of both the dorsal and ventral side of the proximal nail fold forms a gradually desquamating tissue that seals the nail cul-de-sac.
Nail matrix (nail root): The matrix (germinative matrix) is the epithelial structure beneath the nail, starting at the most proximal reach of the nail and finishing at the edge corresponding to the edge of the lunula.
Lunula (half moon): The convex margin of the matrix seen through the nail. It is more pale than adjacent nail bed. It is most commonly visible on the thumb and great toe. It may be concealed by the proximal nail fold.
Nail bed (sterile matrix): The vascular bed upon which the nail rests extending from the lunula to the hyponychium. This is the major territory seen through the nail plate.
Onychodermal band: The onychocorneal band represents the first barrier to penetration of materials to beneath the nail plate. Disruption of this barrier by disease or trauma precipitates a range of further events affecting the nail bed. The white appearance of the central band represents the transmission of light from the digit tip through the stratum corneum and up through the nail. If the digit is placed against a black surface, the band appears dark.
Hyponychium: The cutaneous margin underlying free nail, bordered distally by the distal groove.
Distal groove (limiting furrow): A cutaneous ridge demarcating the border between subungual structures and the finger pulp.

Embryology

Morphogenesis (Figs 3–5)

8–12 weeks
Individual digits are discernible from the eighth week of gestation [1]. The first embryonic element of the nail unit is the nail anlage present from 9 weeks as the epidermis overlying the dorsal tip of the digit.

13–14 weeks
By 13 weeks the nail field is well defined in the finger, with the matrix primordium underlying a proximal nail fold. By 14 weeks the nail plate is seen emerging from beneath the proximal nail fold, with elements arising from the lunula as well as more proximal matrix.



Figure 3 Embryogenesis of the nail apparatus.

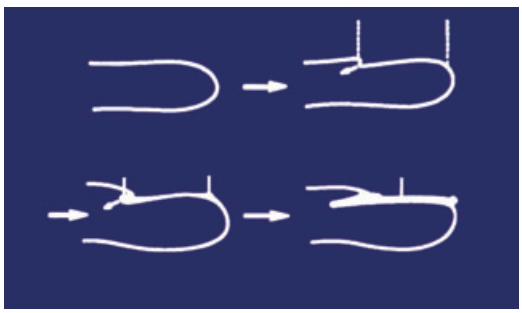


Figure 4 A wedge of matrix primordium moves proximally with the invagination of the proximal nail fold above.

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

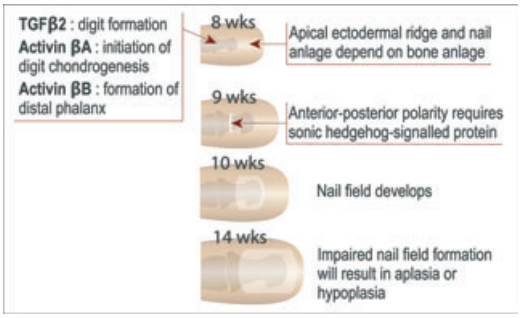


Figure 5 Development of the nail field (Courtesy of E. Haneke).

17 weeks to birth

At 17 weeks, the nail plate covers most of the nail bed. From 20 weeks, the nail unit and finger grow in tandem, with the nail plate abutting the distal ridge. By birth the nail plate extends to the distal groove, which becomes progressively less prominent. The nail may curve over the volar surface of the finger. It may also demonstrate koilonychia. This deformity is normal in the very young and a function of the thinness of the nail plate. It reverses with age.

Regional anatomy and histology

Histological preparation

High quality histological sections of the nail unit are difficult to obtain. The microtome tends to skip on the block and the sections are prone to folding. Softening the nail plate can be attempted to improve sections. There is no universally agreed method for this and many laboratories persevere with routine skin techniques, taking multiple sections to increase the chance of getting something interpretable [6].

Nail softening techniques

Nail alone

1. Lewis [1] recommended routine fixation in 10% formalin and processing as usual. Earlier methods employed used fixation with potassium bichromate, sodium sulphate and water. The section is then decalcified with nitric acid and embedded in collodion.
2. Alkiewicz and Pfister [7] recommended softening the nail with thioglycollate or hydrogen peroxide. Nail fragments are kept in 10% potassium thioglycollate at 37 °C for 5 days or in 20–30% hydrogen peroxide for 5–6 days. The nail is then fixed by boiling in formalin for 1 min before cutting 10–15 mm sections.
3. Suarez *et al.* [8] suggest soaking the clipping for 2 days in a mix of mercuric chloride, chromic acid, nitric acid and 95% alcohol. The specimen is then transferred to absolute alcohol, xylene, successive paraffin mixtures, sectioned at 4 mm and placed on gelatinized slides.
4. An alternative method, described for preserving histological detail in the nail plate, entails fixation in a mix of 5% trichloracetic acid and 10% formalin for the initial 24 h [9]. This is followed by a modified polyethylene glycol-pyroxylin embedding method.

Nail and soft tissue

Nail biopsies containing skin and subcutaneous tissue are not commonly used in cosmetic science. Where there is overlap with clinical medicine such biopsies may be taken and in that instance, more gentle methods of preparation are necessary to avoid damage to the soft tissue element of the biopsy. The specimen can be soaked in distilled water for a few hours before placing in formalin [10]. Good results are obtained with routine fixation and embedding if permanent wave solution, thioglycollate or 10% potassium hydroxide solution, is applied with a cotton swab to the surface of the paraffin block every two or three sections. Lewin *et al.* [11] suggested applying 1% aqueous polysorbate 40 to the cut surface of the block for 1 h at 4 °C.

Sections will sometimes adhere to normal slides, but when there is nail alone, the material tends to curl as it dries and may fall off. This means that it may be necessary to use gelatinized or 3-aminopropyltriethoxysilane slides. Given the difficulty in obtaining high quality sections it is worth cutting many at different levels to maximize the chance of getting what you need.

Routine staining with haematoxylin and eosin is sufficient for most cases. Periodic acid Schiff and Grocott's silver stain can be used to demonstrate fungi; a blancophore fluorochromation selectively delineates fungal walls [12]. Toluidine blue at pH5 allows better visualization of the details of the nail

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

plate [13, 14]. Fontana's argentaffin reaction demonstrates melanin. Haemoglobin is identified using a peroxidase reaction. Prussian Blue and Perl stains are not helpful in the identification of blood in the nail. They are specific to the haemosiderin product of haemoglobin breakdown caused by macrophages. This does not occur in the nail [15, 16].

Masson-Goldner's trichrome stain is very useful to study the keratinization process and Giemsa stain reveals slight changes in the nail keratin.

Polarization microscopy shows the regular arrangement of keratin filaments and birefringence is said to be absent in disorders of nail formation such as leuconychia.

Nail matrix and lunula

For simplicity, the nail matrix (syn. intermediate matrix) will be defined as the most proximal region of the nail bed extending to the lunula (Fig. 6). This is commonly considered to be the source of the bulk of the nail plate, although further contributions may come from other parts of the nail unit (qv. nail growth). Contrast with these other regions helps characterize the matrix.

The matrix is vulnerable to surgical and accidental trauma; a longitudinal biopsy of greater than 3 mm width is likely to leave a permanent dystrophy [17]. Once matrix damage has occurred, it is difficult to effectively repair it [18, 19]. This accounts for the relatively small amount of histological information on normal nail matrix.

It is possible to make distinctions between distal and proximal matrix on functional grounds, given that 81% of cell numbers in the nail plate are provided by the proximal 50% of the nail matrix [20] and surgery to distal matrix is less likely to cause scarring than more proximal surgery. High resolution magnetic resonance imaging identifies the matrix and dermal zones beneath. Baran and Haneke [16] described a zone beneath the distal matrix where there is loose connective tissue and a dense microvascular network. It may be the presence of this network that accounts for the variable sign of red lunulae in some systemic conditions [22, 23].

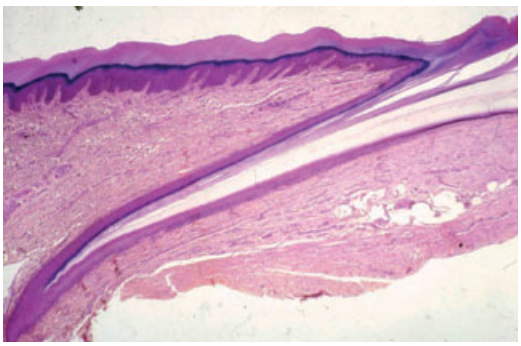


Figure 6 Proximal nail groove and matrix (proximal and intermediate).

Macroscopically, the distal margin of the matrix is convex and is easily distinguished from the contiguous nail bed once the nail is removed, even if the difference is not clear prior to avulsion. The nail bed is a more deep red and has surface corrugations absent from the matrix. At the proximal margin of the matrix, the contour of the lunula is repeated. At the lateral apices, a subtle ligamentous attachment has been described, arising as a dorsal expansion of the lateral ligament of the distal interphalangeal joint [24]. Lack of balance between the symmetrical tension on these attachments may explain some forms of acquired and congenital malalignment [25].

Clinically, the matrix is synonymous with the lunula, or half moon, which can be seen through the nail emerging from beneath the proximal nail fold as a pale convex structure. This is most prominent on the thumb, becoming less prominent in a gradient towards the little finger. It is rarely seen on the toes. The absence of a clinically identifiable lunula may mean that the vascular tone of the nail bed and matrix have obscured it or that the proximal nail fold extends so far along the nail plate that it lies over the entire matrix. Cosmetically, there is significance to the lengths of the lunula and proximal nail fold. A long nail fold provides protection to the matrix and makes it less vulnerable to irritants and trauma. However, aesthetic norms mean that there is often a preference for a large lunula. This means that manicure can entail cutting back of the cuticle and pushing back of the nail fold. Such manicure not only makes the matrix more likely to suffer from damaging influences, but represents such an influence itself, where transverse markings make be seen in the nail as a result [26].

Routine histology

The cells of the nail matrix are distinct from the adjacent nail bed distally and the ventral surface of the nail fold, lying at an angle above. The nail matrix is the thickest area of stratified squamous epithelium in the midline of the nail unit, comparable

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

with the hyponychium. There are long rete ridges characteristically descending at a slightly oblique angle, their tips pointing distally. Laterally, the matrix rete ridges are less marked, whereas those of the nail bed nail folds become prominent.

Unlike the overlying nail fold, but like the nail bed, the matrix has no granular layer (Fig. 7). The demarcation between overlying nail fold and matrix is enhanced by the altered morphology of the rete ridges. At their junction at the apex of the matrix and origin of the nail, the first matrix epithelial ridge may have a bobbed appearance like a lopped sheep's tail. Periodic acid Schiff staining is marked at both the distal and proximal margins of the intermediate matrix.

Distally, there is often a step reduction in the epithelial thickness at the transition of the matrix with the nail bed. This represents the edge of the lunula.

Nail is formed from the matrix as cells become larger and more pale and eventually the nucleus disintegrates. There is progression with flattening, elongation and further pallor. Occasionally retained shrunken or fragmented nuclei persist to be included into the nail plate.

Melanocytes are present in the matrix where they reach a density of up to 300 mm^2 [27–31]. They are dendritic cells found in the epibasal layers and most prominent in the distal matrix [29–31]. They occupy two compartments in the matrix. The first is a compartment of quiescent melanocytes unable to synthesize melanin under normal conditions. These amelanotic melanocytes (DOPA-negative) have been demonstrated by immunohistochemical techniques: this reacts with the monoclonal (Mo) antibodies (Ab) TMH1 and MoAb B8G3 which recognize tyrosinase-related protein (TRP) 1 but not with MoAb 5C12 and MoAb 2B7 which recognize tyrosinase.

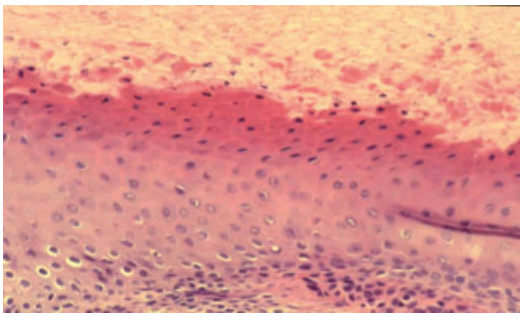


Figure 7 Stratum granulosum is missing in the nail matrix.

The second is of functionally differentiated melanocytes which are DOPA positive [28]. Immunohistochemical studies [29, 31] have demonstrated that these melanocytes contain a multifunctional enzyme tyrosinase, which catalyses the initial events of melanogenesis and the other regulatory proteins, known as TRP1 and 2. These melanocytes consequently express all the key enzymes necessary to melanin synthesis. In contrast to previously held views [29, 32] the melanocytes of the distal matrix are not more numerous than those of the proximal matrix. Their density is nevertheless low 217 mm^2 [31] compared to that of the epidermis. The difference between these matricial zones is solely in the distribution of dormant and potentially active compartments. The melanocytes of the proximal matrix are mainly dormant. In the distal matrix the quiescent melanocytic compartment is reduced while the group of functionally differentiated melanocytes is dominant.

The melanocytes of both divisions, unlike the epidermal melanocytes, express MoAb HMB45 which recognize the glycoprotein Pmel 17 present in the immature melanosome.

The constellation of these immunohistochemical data suggests that the matrix melanocytes contain premelanosomes and melanosomes I and II with little or no active synthesis of mature melanosomes.

This is in accordance with ultrastructural studies [28] which demonstrated the scarcity of recognizable melanosomes in the Caucasian nail.

Perrin *et al.* [31] have also defined a smaller population of nail bed-dormant melanocytes, with approximately 25% of the number of melanocytes formed in the matrix (Fig. 4). This differs from de Berker *et al.*'s observation [30] where the nail bed was noted to lack melanocyte markers. This discrepancy may be due to differing investigation techniques, one of which was the split-skin preparation.

There is only one ultrastructural study comparing the nail melanocytes according to the skin colour type [33]. The melanocytes in Japanese nails contain all gradation of maturing melanosomes (the majority being of an immature variety) and transferred melanosomes (often just half-filled with melanin) are regularly seen within the keratinocytes. In black subjects, most of the melanosomes including transferred melanosomes are mature.

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

Intraepithelial distribution of the melanocytes in eponychium, matrix and nail bed

Melanocytes of the proximal matrix and, to a lesser degree, of the distal one have a basal as well as suprabasal distribution [27, 29, 31]. These false images of migration are explained by the thickness of the basal keratinocytic compartment composed of 2 to 10 layers of basaloid cells. This suprabasal location can rise very high, immediately beneath the keratogenous zone and such dendritic melanocytes, expressing HMB45, may pose a problem of interpretation where there is a suspicion of melanoma.

The melanocytes of the hyponychium and of the nail bed retain a basal distribution because of the monocellularity of the basal layer in these two anatomical regions.

Melanin in the nail plate is composed of granules derived from matrix melanocytes [34]. Longitudinal melanonychia may be a benign phenomenon, particularly in Afro-Caribbeans – 77% of black people will have a melanonychia by the age of 20 and almost 100% by 50 [35, 36]. The Japanese also have a high prevalence of longitudinal melanonychia, being present in 10–20% of adults [37]. In a study of 15 benign melanonychia in Japanese patients, they were found to arise from an increase in activity and number of DOPA-positive melanocytes in the matrix, not a melanocytic naevus [27]. Longitudinal melanonychia in Caucasians is more sinister. Oropeza [38] stated that a subungual pigmented lesion in this group has a higher chance of being malignant than of being benign.

There is only a thin layer of dermis dividing the matrix from the terminal phalanx. This has a rich vascular supply (see below) and an elastin and collagen infrastructure giving attachment to periosteum.

Electron microscopy

Transmission electron microscopy confirms that in many respects, matrix epithelium is similar to normal cutaneous epithelium [33, 39–41]. The basal cells contain desmosomes and hemidesmosomes and interdigitate freely. Differentiating cells are rich in ribosomes and polysomes and contain more RNA than equivalent cutaneous epidermal cells. As cell differentiation proceeds towards the nail plate, there is an accumulation of cytoplasmic microfibrils (7.5–10 nm). These fibrils are haphazardly arranged within the cells up to the transitional zone. Beyond this, they become aligned with the axis of nail plate growth.

Membrane-coating granules (Odland bodies) are formed within the differentiating cells. They are discharged onto the cell surface in the transitional zone and have been thought to contribute to the thickness of the plasma membrane. They may also have a role in the firm adherence of the squamous cells within the nail plate, which is a notable characteristic [42]. The glycoprotein characteristics of cell membrane complexes isolated from nail plate may reflect the constituents of these granules [43].

Nail bed and hyponychium

The nail bed extends from the distal margin of the lunula to the hyponychium [44]. Avulsion of the nail plate reveals a pattern of longitudinal epidermal ridges stretching to the lunula. On the underside of the nail plate is a complementary set of ridges, which has led to the description of the nail being led up the nail bed as if on rails (Fig. 8).



Figure 8 The epidermis of the nail bed has longitudinal ridges visible after nail avulsion.

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

The small vessels of the nail bed are orientated in the same axis. This is demonstrated by splinter haemorrhages (Fig. 9), where haem is deposited on the undersurface of the nail plate and grows out with it. The free edge of a nail loses the ridges, suggesting that they are softer than the main nail plate structure. The nail bed also loses these ridges shortly after loss of the overlying nail. It is likely that the ridges are generated at the margin of the lunula on the ventral surface of the nail to be imprinted upon the nail bed.

The epidermis of the nail bed is thin over the bulk of its territory. It becomes thicker at the nail folds where it develops rete ridges. It has no granular layer except in disease states. The dermis is sparse, with little fat, firm collagenous adherence to the underlying periosteum and no sebaceous or follicular appendages [45]. Sweat ducts can be seen at the distal margin of the nail bed using *in vivo* magnification [46].

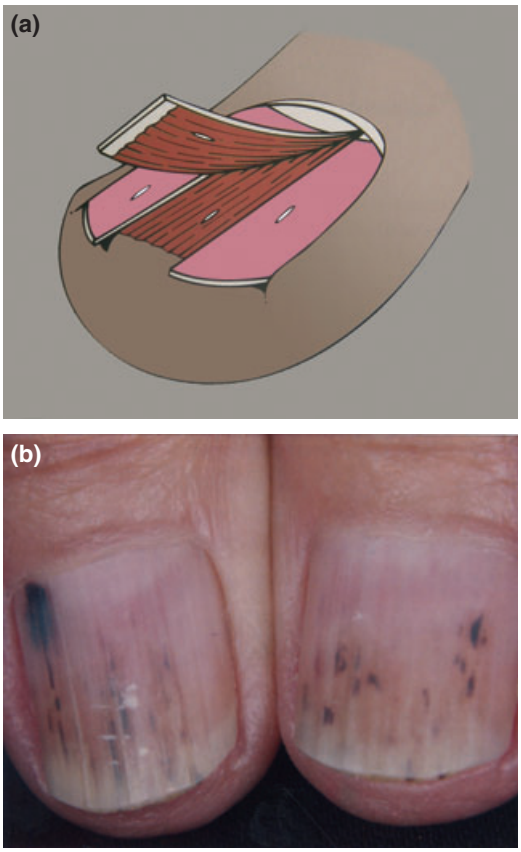


Figure 9 (a) The appearance of splinter haemorrhages. Haem from longitudinal nail bed vessels is deposited on the underside of the nail plate this grows out in the shape of a splinter. (b) Splinter haemorrhages.

The hyponychium lies between the distal ridge and the nail plate and represents a space as much as a surface (Fig. 10). The hyponychium and onychocorneal band may be the focus or origin of subungual hyperkeratosis in some diseases and as a reaction to trauma and fungal infection (Table I).

The hyponychium and overhanging free nail provide a crevice. This is a reservoir for microbes, relevant in surgery and the dissemination of infection. After 10 min of scrubbing the fingers with povidone iodine, nail clippings were cultured for bacteria, yeasts and moulds [47]. In 19 of 20 patients *Staphylococcus epidermidis* was isolated, seven patients had an additional bacteria, eight had moulds and three had yeasts. These findings could have significance to both surgeons and patients. There is a literature on the potential for cosmetically enhancing nails of health professionals to harbour bacteria. This includes long nails and nails with extensions, but does not appear related to nail lacquer alone [48].

The hand to mouth transfer of bacteria is suggested by the high incidence of *Helicobacter pylori* beneath the nails of those who are seropositive for antibodies and have oral carriage. Dowsett *et al.* [49] found that 58% of those with tongue *H. pylori* had it beneath the index fingernail, representing a significant ($P = 0.002$) association.

Nail folds

The proximal and lateral nail folds give purchase to the nail plate by enclosing more than 75% of its periphery. They also provide a physical seal against the penetration of materials to vulnerable subungual and proximal regions.

The epidermal structure of the lateral nail folds is unremarkable, and comparable with normal skin. There is a tendency to hyperkeratosis,

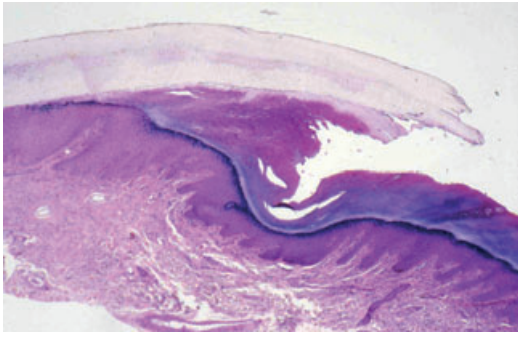


Figure 10 Hyponychium space.

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

Table I Clinical appearance of distal zones of the nail bed

Zone	Sub-zone	Appearance
Free edge		Clear grey
Onychocorneal band		
I	Distal pink zone	0.5–2 mm distal pink margin, may merge with free edge
II	Central white band	0.1–1 mm distal white band representing the point of attachment of the stratum corneum arising from the digit pulp
III	Proximal pink gradient	Merging with nail bed

Source: Ref. [2].

sometimes associated with trauma. When the trauma arises from the ingrowth of the nail, considerable soft tissue hypertrophy can result, with repeated infection (qv. ingrowing nails).

The proximal nail fold has significance in four main areas:

1. It may contribute to the generation of nail plate through a putative dorsal matrix on its ventral aspect.
2. It may influence the direction of growth of the nail plate by directing it obliquely over the nail bed.
3. Nail fold microvasculature can provide useful information in some pathological conditions.
4. When inflamed it can influence nail plate morphology as seen in eczema, psoriasis, habit tic deformity and paronychia.

The first two issues are dealt with in the section on nail growth (see 'Nail growth' below), the latter under vasculature (see 'Vascular supply' below) and dermatological diseases and the nail.

Immunohistochemistry of periungual tissues

Keratins

The most extensive immunohistological investigations of the nail unit have utilized keratin antibodies. The nail plate [50, 51], human embryonic nail unit [51–53], accessory digit nail unit [5, 6] and adult nail unit [53, 54, 56] have all been examined.

Using monospecific antibodies, de Berker *et al.* [55] detected keratins 1 and 10 in a suprabasal location in the matrix and noted their absence from the nail bed (Fig. 11). The absence of keratin 10 from the nail bed was confirmed by Perrin *et al.* [56] (see 'Nail growth' above and 'Nail plate' below). Keratins 1 and 10 are 'soft' epithelial keratins found suprabasally in normal skin [57] and characteristic of cornification with terminal keratinocyte differentiation. Their absence from normal nail bed is reversed in disease where nail bed cornification is often seen, alongside development of a granular layer and expression of keratins 1 and 10 [58]. The development of a granular layer in subungual tissues can be interpreted as a pathological sign in nail histology, seen in a range of diseases and probably associated with changes in keratin expression [59].

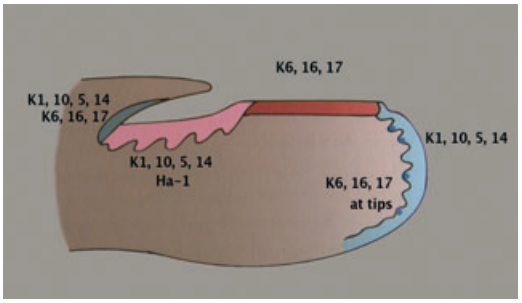


Figure 11 Distribution of keratins in the human periungual and subungual tissues.

Ha-1 is found in the matrix. Ha-1 is a 'hard' keratin detected by the monoclonal anti-keratin antibody LH TRIC 1, is limited to the matrix of the nail and the germinal matrix of the hair follicle [55, 60]. Keratin 19 is probably not found in the adult matrix [52, 55]. However, Moll *et al.* [52] did detect keratin 19 at this site in 15-week embryo nail units. Keratin 19 is also found in the outer root sheath of the hair follicle and lingual papilla [51]. Perrin *et al.* [56] used monospecific antibodies to the hard keratins hHa2, hHb2, hHa5, hHb5, hHa1, hHb1, hHb6, hHa4 and hHa8. The presence of hHa1, hHb1, hHb6 and hHa4 were all confirmed in emerging sequence

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

from the basal layers of the matrix upwards into the nail plate. Small numbers of cells in the apex of the matrix expressed hHa8, and hHb5 was seen co-expressed with K5 and K10 in the uppermost layers of the basal compartment of the matrix. *In vitro* experimentation with nail matrix dermis illustrates that it is able to induce hard 'nail-like' keratin in non-matrix epidermis [61].

The co-localization of hard and soft keratins within single cells of the matrix has been observed by several workers in bovine hoof [62] and human nail [55, 63, 64], suggesting that these cells are contributing both forms of keratin to the nail plate. This dual differentiation continues into *in vitro* culture of bovine hoof matrix cells [63]. Culture of human nail matrix confirms the persistence of hard keratin expression [65, 66].

Markers for keratins 8 and 20 are thought to be specific to Merkel cells in the epidermis. Positive immunostaining for these keratins has been noted by Lacour *et al.* [53] in adult nail matrix and de Berker *et al.* [55] in infant accessory digits. Some workers have failed to detect Merkel cells and although it seems likely that they are present in fetal and young adult matrix, it may be that the cells are less common or absent as people age [67].

The nail bed appears to have a distinct identity with respect to keratin expression. Keratins 6, 16 and, to a lesser degree, 17 are all found in the nail bed and are largely absent from the matrix [6]. This finding has gained clinical significance with the characterization of the underlying fault in some variants of pachyonychia congenita where abnormalities of nail bed keratin lead to a grossly thickened nail plate. Mutations in the gene for keratin 17 have been reported in a large Scottish kindred with the PC-2, or Jackson-Lawlor, phenotype [68, 69]. There is a cross-over with steatocystoma multiplex where the same mutation of keratin 17 may cause this phenotype which appears to be independent of the specific keratin 17 mutation [70–72]. Mutations in the gene coding for K6b produce a phenotype seen with K17 gene mutations [73]. Mutations in the K6a [74] and K16 [69] genes have been reported in PC-1, originally described as the Jadassohn–Lewandowsky variant of pachyonychia congenita.

Expression of keratins 6, 16 and 17 extend beyond the nail bed onto the digit pulp and are thought to match the physical characteristics of this skin which is adapted to high degrees of physical stress [75]. In particular, expression of keratin 17 is found at the base of epidermal ridges, which might also support the idea that this keratin is associated with stem cell function.

Non-keratin immunohistochemistry

Haneke [76] has provided a review of other important immunohistochemically detectable antigens. Involucrin is a protein necessary for the formation of the cellular envelope in keratinizing epithelia. It is strongly positive in the upper two-thirds of the matrix and elsewhere in the nail unit [77] and weakly detected in the suprabasal layers. Pancornulin and sciellin are also detected in the matrix [77]. The antibody HHF35 is considered specific to actin. It has been found to show a strong membranous staining and weak cytoplasmic staining of matrix cells [76].

In the dermis, vimentin was strongly positive in fibroblasts and vascular endothelial cells. Vimentin and desmin were expressed in the smooth muscle wall of some vessels. S100 stain, for cells of neural crest origin, revealed perivascular nerves, glomus bodies and Meissner's corpuscles distally.

Filaggrin could not be demonstrated in the matrix in Haneke's work or by electron microscopy [51]. However, Manabe and O'Guin [78] have detected the co-existence of trichohyalin and filaggrin in monkey nail, located in the area they term the 'dorsal matrix' which is likely to correspond to the most proximal aspect of the human nail matrix as it merges with the undersurface of the proximal nail fold. Kitahara and Ogawa [64] have identified filaggrin in the human nail in the same location and O'Keefe *et al.* [79] have found trichohyalin in the 'ventral matrix' of human nail, which is synonymous with the nail bed. Manabe and O'Guin noted that these two proteins coexist with keratins 6 and 16, which are more characteristic of nail bed than matrix. It is argued that filaggrin and trichohyalin may act to stabilize the intermediate filament network of K6 and K16, which are normally associated with unstable or hyperproliferative states.

The plasminogen activator inhibitor, PAI-Type 2, has been detected in the nail bed and matrix where it has been argued that it may have a role in protecting against programmed cell death [80].

The basement membrane zone of the entire nail unit has been examined, employing a wide range of monoclonal and polyclonal antibodies [54].

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

Collagen VII, fibronectin, chondroitin sulphate and tenascin were among the antigens detected. All except tenascin were present in a quantity and pattern indistinguishable from normal skin. Tenascin was absent from the nail bed, which was attributed to the fact that the dermal papillae are altered or considered absent (Table II).

Nail plate

The nail plate is composed of compacted keratinized epithelial cells. It covers the nail bed and intermediate matrix. It is curved in both the longitudinal and transverse axes. This allows it to be embedded in nail folds at its proximal and lateral margins, which provide strong attachment and make the free edge a useful tool. This feature is more marked in the toes than the fingers. In the great toe, the lateral margins of the matrix and nail extend almost half way around the terminal phalanx. This provides strength appropriate to the foot.

The upper surface of the nail plate is smooth and may have a variable number of longitudinal ridges that change with age. These ridges are sufficiently specific to allow forensic identification and the distinction between identical twins [81]. The ventral surface also has longitudinal ridges that correspond to complementary ridges on the upper aspect of the nail bed (see 'Nail bed' above) to which it is bonded. These nail ridges may be best examined using polarized light. They can also be used for forensic identification [82].

The nail plate gains thickness and density as it grows distally [83] according to analysis of surgical specimens. *In vivo* ultrasound suggests that there may be an 8.8% reduction in thickness distally [84]. A thick nail plate may imply a long matrix. This stems from the process whereby the longitudinal axis of the matrix becomes the vertical axis of the nail plate (Fig. 12). Other factors, like linear rate

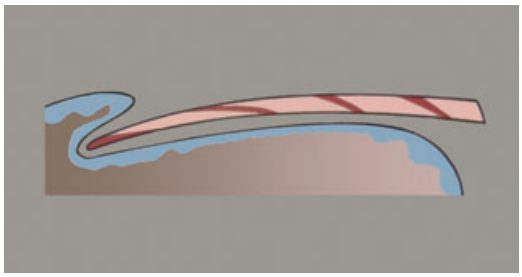


Figure 12 Shade areas represent 7-day periods of nail growth, separated by 1 month with transition of nail from horizontal to oblique axis over 4 months.

Table II Analysis of nail unit basement membrane zone using monoclonal and polyclonal antibodies

	Digit 1: Nail apparatus					Digit 2: Nail apparatus				Digit 3	
	Fold	Matrix	Bed	HN	Proximal palangeal skin	Fold	Matrix	Bed	HN	Split skin	Intact skin
Mono. Ab											
LH7:2	+	+	+	+	+	+	+	+	+	epi	+
L3d	+	+	+	+	+	+	+	+	+	epi	+
Co1 IV	+	+	+	+	+	+	+	+	+	epi	+
GB3	+	+	+	+	+	+	+	+	+	epi	+
LH24	+	+	+	+	+	+	+	+	+	epi	+
LH39	+	+	+	+	+	+	+	+	+	epi	+
GDA	+	+	+	+	+	+	+	+	+	epi	+
Tenascin	+	+	+	+	+	+	+	+	+	epi	+
a6	+	+	+	+	+	+	+	+	+	epi	+
G71	+	+	+	+	+	+	+	+	+	epi	+
Poly. Ab											
Fibronectin	–	–	–	–	–	–	–	–	–	–	–
Laminin	+	+	+	+	+	+	+	+	+	derm	+
BP 220 kDa	+	+	+	+	+	+	+	+	+	epi	+
EBA 250 kDa	+	+	+	+	+	+	+	+	+	derm	+
LAD 285 kDa	+	+	+	+	+	+	+	+	+	epi	+
LAD ? kDa	+	+	+	+	+	+	+	+	+	derm	+

HN, hyponychium. *Source*: Ref. [2].

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

of nail growth [85], vascular supply, subungual hyperkeratosis and drugs also influence thickness.

Light microscopy

Lewis [1] described a silver stain that delineates the nail plate zones. Three regions of nail plate have been histochemically defined [86]. This appears corroborated by synchrotron X-ray microdiffraction [87]. The dorsal plate has a relatively high calcium, phospholipid and sulphydryl group content. It has little acid phosphatase activity and is physically hard. The phospholipid content may provide some water resistance. The intermediate nail plate has a high acid phosphatase activity, probably corresponding to the number of retained nuclear remnants. There is a high number of disulphide bonds, low content of bound sulphydryl groups, phospholipid and calcium. Controversy allows that the ventral nail plate may be a variable entity [88]. Jarrett and Spearman [86] described it as a layer only one or two cells thick. These cells are eosinophilic and move both upwards and forward with nail growth. With respect to calcium, phospholipid and sulphydryl groups it is the same as the dorsal nail plate. It shares a high acid phosphatase and frequency of disulphide bonds with the intermediate nail plate.

Ultrasound examination of *in vivo* and avulsed nail plates suggests that it has the physical characteristics of a bilamellar structure [89]. There is a superficial dry compartment and a deep humid one. This has been given as evidence against the existence of a ventral matrix contribution to the nail plate.

Germann *et al.* [90] utilized a form of tape-stripping in conjunction with light microscopy to examine dorsal nail plate corneocyte morphology in disease and health. They found that conditions of rapid nail growth (psoriasis and infancy) resulted in smaller cell size. Sonnex *et al.* [91] describes the histology of transverse white lines in the nail.

Electron microscopy

Scanning electron microscopy clarifies basic nail plate structure [92, 93]. It has added to our understanding of onychoschizia, also known as lamellar splitting, where the free edge of the nail develops a patterns of transverse splitting [94, 95]. In the normal nail corneocytes can be seen adherent to the dorsal aspect of the nail plate. In cross-section, the compaction of the lamellar structure is visible. Both these features can be seen to be disrupted in onychoschizia following repeated immersion and drying of the nail plates. Limited electron microscopic studies have also been undertaken on the pattern of fungal invasion of the nail plate [96, 97]. Scanning electron microscopy can also be used on 'casts' made from an amputated digit in which the blood supply has been injected with a resistant material. In this way, dissolving the surrounding tissues allows electron microscopy to reveal the full pattern of microvasculature in different regions of the nail unit [98].

Transmission electron microscopy (Figs 13–20) has been used to identify the relationship between the corneocytes of the nail plate [99]. Using Thierry's tissue processing techniques, material for the following description has been provided. Cell membranes and intercellular junctions are easily discernible. Even though at low magnification one can differentiate the dorsal and intermediate layers of the nail plate, the exact boundary is unclear using transmission electron microscopy. Cells on

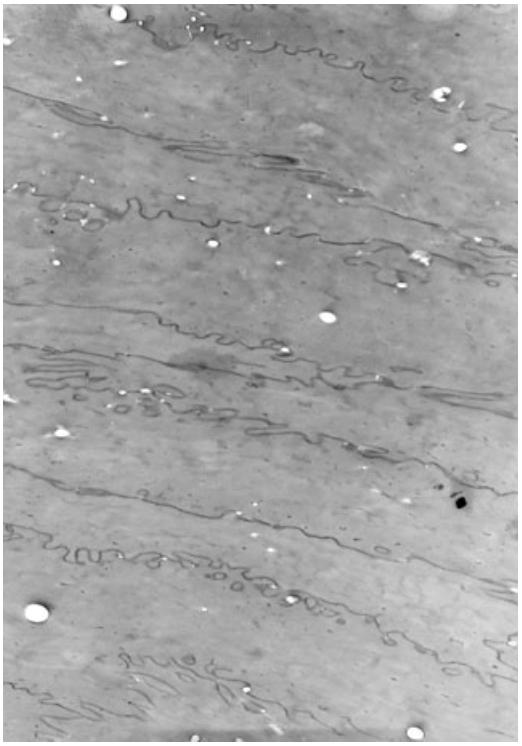


Figure 13 Transmission electron microscopy of the dorsal nail plate. The onychocytes, are flattened, with discretely indented cell membranes (original magnification ×5000).

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

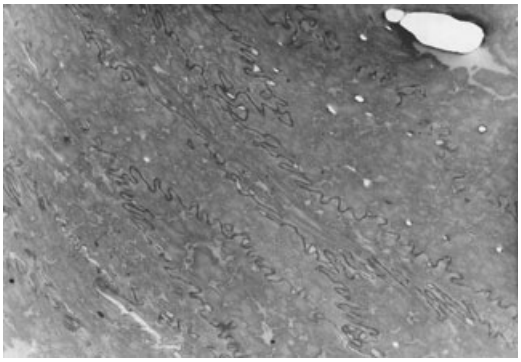


Figure 14 Transmission electron microscopy of the dorsal nail plate. The onychocytes are flattened and separated by ampullar dilatations (original magnification ×6300).

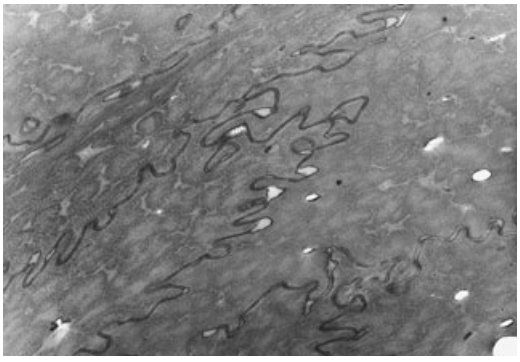


Figure 15 Transmission electron microscopy of the dorsal nail plate. Ampullar dilatations between onychocytes (original magnification ×10.000).

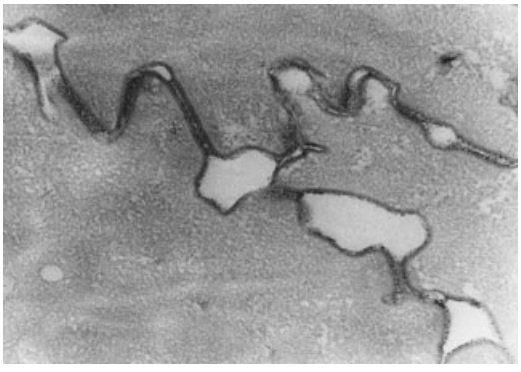


Figure 16 Transmission electron microscopy of the dorsal nail plate. Intercellular junctions in the dorsal nail plate.



Figure 17 Transmission electron microscopy of the ventral nail plate. The onychocytes are thicker, with indented cell membranes (original magnification ×5000).



Figure 18 Transmission electron microscopy of the ventral nail plate. The onychocytes have indented cell membranes forming anchoring knots (original magnification ×10 000).

the dorsal aspect are half as thick as ventral cells with a gradation of sizes in between. In the dorsal nail plate, large intercellular spaces are present corresponding to ampullar dilatations. These gradually diminish in the deeper layers and are absent in the ventral region. At this site, cells are

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

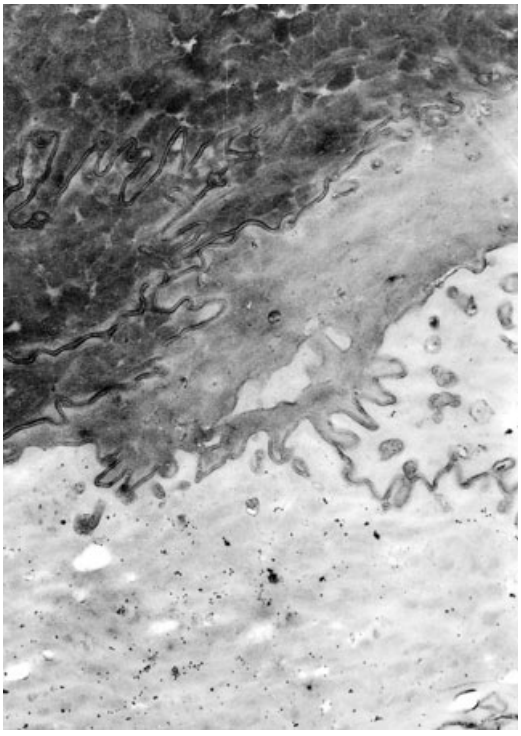


Figure 19 Transmission electron microscopy at the junction between the ventral nail plate and the subungual keratin. Onychocytes sending our digitations penetrating the subungual corneocytes.

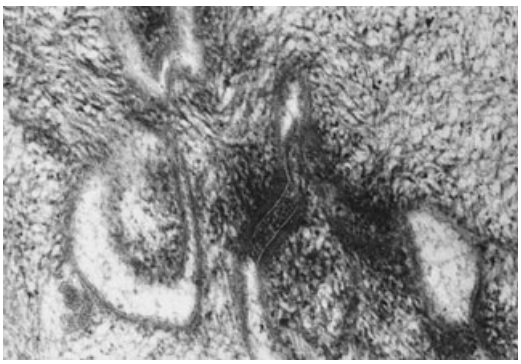


Figure 20 Desmosome in the hyponychial keratin.

joined by complete folds, membranes of adjacent cells appearing to penetrate each other to form 'anchoring knots'.

Corneocytes of the dorsal nail plate are joined laterally by infrequent deep interdigitations. The plasma membranes between adjacent cell layers are more discretely indented, often with no invaginations. Nail bed cells show considerable infolding and interdigitation at their junction with the nail plate cells. They are polygonal and show no specific alignment. They are between 6 and 20 mm across and show neither tight nor gap junctions. They do, however, have desmosomal connections of the type seen in normal epidermis.

Using different preparation techniques, other workers have demonstrated other anatomical details. On the cytoplasmic side of the cell membranes of nail plate cells lies a layer of protein particles [39–101]. Other staining techniques suggest that the single type of intercellular bond described by Parent *et al.* [99] may be a spot desmosome [102].

Vascular supply

The nail fold capillary network is seen easily with a ×4 magnifying lens, ophthalmoscope or dermatoscope [103, 104]. A dermatoscope is a routine instrument in clinical dermatological. The vascular pattern is similar to the normal cutaneous plexus in health, except that the capillary loops are more horizontal and visible throughout their length. The loops are in tiers of uniform size, with peaks equidistant from the base of the cuticle (Fig. 21) [105]. The venous arm is more dilated and tortuous than the arterial arm. There is a wide range of morphologies within the normal population [106]. Features in some disorders may be sufficiently gross to be useful without magnification; erythema and haemorrhages being the most obvious.

Intravenous bolus doses of Na-fluorescein dye have been followed through nail fold microscopy [107]. There is rapid and uniform leakage from the capillaries in normal subjects to within 10 mm of the capillaries. It is suggested that a sheath of collagen may prevent diffusion beyond this point. The same procedure has been followed in patients with rheumatoid arthritis demonstrating decreased flow rates and abnormal flow patterns, but no change in vessel leakage [108]. Videomicroscopy [109] and laser Doppler [110] can be used to measure dynamic elements of the nail fold capillary function.

Glomus bodies

A glomus body is an end-organ apparatus in which there is an arteriovenous anastomosis (AVA) bypassing the intermediary capillary bed. AVAs are connections between the arterial and venous side of the circulation with no intervening capillaries. Each glomus body is an encapsulated oval organ 300 mm long composed of a tortuous

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie



Figure 21 Capillary loops in the proximal nail fold.

vessel uniting an artery and venule, a nerve supply and a capsule. Digital nail beds contain 93–501 glomus bodies per cm^3. They lie parallel to the capillary reservoirs which they bypass. They are able to contract asynchronously with their associated arterioles such that in the cold, arterioles constrict and glomus bodies dilate. They are particularly important in the preservation of blood supply to the peripheries in cold conditions.

Nerve supply

The periungual soft tissues are innervated by dorsal branches of paired digital nerves (Fig. 22). Wilgis and Maxwell [111] stated that the digital nerve is composed of three major fascicles

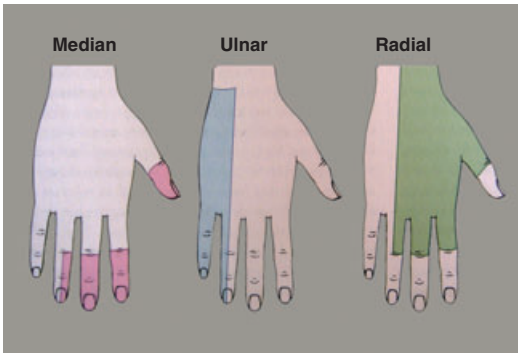


Figure 22 Periungual soft tissue innervation.

supplying the digit tip, with the main branch passing under the nail bed and innervating both nail bed and matrix [112]. Winkelmann [113] showed many nerve endings adjacent to the epithelial surface, mainly in the nail folds.

Comparative anatomy and function

The comparative anatomy of the nail unit can be considered with other ectodermal structures and most particularly hair and its follicle. The human nail can be considered to have many mechanical and social functions, the most prominent of which are:

- fine manipulation;
- scratching;
- physical protection of the extremity;
- a vehicle for cosmetics and aesthetic manipulation.

However, among its multiple functions, the nail provides counterpressure to the pulp that is essential to the tactile sensation involving the fingers and to the prevention of the hypertrophy of the distal wall tissue produced after nail loss of the great toe nail.

For many of these functions there is therefore an optimal nail length, although there can be conflict between aesthetic and functional norms. Testing grip strength and dexterity with typing is one way of determining the effect of nail length on function. Jansen *et al.* [114] demonstrated a significant loss of grip power and typing speed when extending nails from 0.5 cm at the free edge to 1 and 2 cm.

The nail and other appendages

An appendage is formed through the interaction of mesoderm and ectoderm, which in differentiated

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

states usually means the interaction between dermis and epidermis. These appendages most closely related to nail include hair and tooth. There are many shared aspects of different appendages, illustrated by diseases, morphology and analysis of the biological constituents.

Achten [115] noted that the nail unit was comparable in some respects to a hair follicle, sectioned longitudinally and laid on its side. The hair bulb was considered analogous to the intermediate nail matrix and the cortex to the nail plate. As a model to stimulate thought, this idea is helpful. It also encourages the consideration of other manipulations of the hair follicle that might fit the analogy more tightly. The nail unit could be seen as an unfolded form of the hair follicle, producing a hair with no cortex, just hard cuticle. Scanning electron microscopy of the nail confirms that its structure is more similar to compacted cuticular cells than cortical fibres. A third model could represent the nail unit as a form of follicle abbreviated on one side, providing a modified form of outer root sheath to mould and direct nail growth in the manner of the proximal nail fold. The matrix and other epithelial components of tooth can be seen in a similar comparative light and even the lingual papilla which shares some keratin expression with the nail, shows some morphological similarities with the nail and hair follicle [116].

Constituents of some appendages, such as keratin and trichohyalin, are both specific in their physical attributes and specific to certain appendages. A considerable amount of biochemical work on hair and nail illustrates this point. In one study [117] two-dimensional electrophoresis was used to determine the presence of nine keratins in human hair and nail. Those of molecular weights 76, 73, 64, 61 and 55 kDa were common to hair and nail. One component of 61 kDa was specific to hair, and two components, both with a molecular weight of 50 kDa, were specific to nail. Further definition of these proteins was given by Heid *et al.* [118] who employed gel electrophoresis, immunoblotting, peptide mapping and complementary keratin binding analysis. Heid *et al.* found that whereas nail plate contained both 'soft' epithelial and 'hard' hair/nail keratins, plucked hairs contained only the latter. By contrast, 'soft' epithelial keratins could be detected in the hair follicle and coexpressed with 'hard' keratins in a pattern also seen in the nail matrix. Although these 'hard' keratins are found in small amounts in the embryonic thymus and lingual papilla, they can generally be thought to be a feature of the hair/nail differentiation. Common ground between the lingual papilla and nail is demonstrated in the nail dystrophy of dyskeratosis congenita, where there are oral changes including changes in expression of keratins of the lingual papillae [119].

The character of the nail plate and hair has led to their use in assays of circulating metabolites. They both lend themselves to this because they are long-lasting structures that may afford historical information. Additionally, their protein constituents bind metabolites and they provide accessible specimens. This allows both hair and nail to be used in the detection of systemic metabolites which may have disappeared from the blood many weeks previously.

Physiology

Nail growth

Definition of the nail matrix

In the first section we have attempted to define the matrix in anatomical terms, assisted by histology and immunohistochemistry illustrating regional differentiation within the nail unit and in particular with respect to keratin expression. There has been a range of studies over the last 50 years attempting to define the matrix in functional as well as anatomical terms. Lewis [120] established the anatomical definition based on silver staining of nail plate, suggesting three zones of matrix function: namely, the classical matrix (intermediate matrix), a proximal portion of the ventral aspect of the proximal nail fold (proximal matrix) and the nail bed (distal matrix). Most contemporary literature accepts the intermediate matrix and being the main source of nail plate and now matrix and intermediate matrix are synonymous. This conclusion has developed through the work below.

Markers of matrix and nail bed proliferation

Zaias and Alvarez [121] disagreed with Lewis on the basis of *in vivo* autoradiographic work on squirrel monkeys, where dynamic aspects of the process were being examined. Tritiated thymidine injected into experimental animals was only incorporated into classical matrix (or intermediate matrix to use Lewis' terminology). Norton used

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

human subjects in further autoradiographic studies [122]. Although there was some incorporation of radiolabelled glycine in the area of the nail bed, it was in a poorly defined location, making clear statements impossible.

More recent immunohistochemical techniques have allowed us to examine proliferation markers in human tissue, without the drawbacks of autoradiography. Antibodies to proliferating cell nuclear antigen, and to the antigen K1–67 associated with cell cycling, have been used on longitudinal sections of healthy and diseased nail units [123]. Both markers demonstrated labelling indices in excess of 20% for the nail matrix in contrast with 1% or less for the nail bed in healthy tissue. In psoriatic nail and onychomycosis, the labelling index of nail bed rises to >29%. Although these indices do not directly measure nail plate production, a very low index for normal nail bed is consistent with other studies suggesting that the nail bed is an insignificant player in normal nail production. The situation may change in disease and the definition of nail plate becomes difficult when substantial subungual hyperkeratosis produces a ventral nail of indeterminate character [124].

Nail plate indicators of matrix location

Johnson *et al.* [125, 126] dismissed the evidence of Zaias and Alvarez, claiming that the methodology was flawed. She examined nail growth by the measurement of change in nail thickness along a proximal to distal longitudinal axis. She demonstrated that 21% of nail plate thickness in traumatically lost big toenails was gained as the nail grew over the nail bed. This was taken as evidence of nail bed contribution to the nail plate.

A similar study developed this observation with histology of the nail plate taken at fixed reference points along the longitudinal nail axis and comparing nail plate thickness at these sites with numbers of corneocytes in the dorso-ventral axis of the nail [20]. The result of this was to confirm the observation that the nail plate thickens over the nail bed but that this is not matched by an increase in nail cells. In fact, the number of cells reduces by 10%, but this was not of statistical significance. These combined studies may be reconciled if we propose that the shape of cells within the big toenail becomes altered with compaction as the nail grows. This is likely where clinical experience shows that the nail develops transverse rippling where there is habitual distal trauma.

If the loss of nail cell numbers along the nail bed is a genuine observation, it might suggest that they are being shed from the nail surface. This is compatible with the status of nail plate as a modified form of stratum corneum. Heikkilä *et al.* [127] provides evidence in support of this where nail growth was measured by making indentations on the nail surface and measuring the change in the volume of these grooves as they reach the free edge. There was a reduction of volume by 30–35%, which was taken as evidence of a nail bed contribution to the nail plate. However, this interpretation is less believable than the possibility that the nail is losing cells from the surface, and histology of grooves in a similar study shows that this is likely to be the case [128].

Ultrasound as a tool to define location

Ultrasound studies of the nail plate have done little to support the notion that the nail bed contributes significantly to its substance [84, 89]. Jemec claimed that the nail plate had a clear two-part structure, none of which appeared to come from the nail bed. Finlay observed that the nail plate had a more rapid ultrasound transmission distally; paradoxical if one imagines a nail bed contribution. This last comment is almost diametrically opposite that of Johnson *et al.* [126].

Clinical markers of matrix location

The clearest demonstration of nail generation is the effect of digit amputation at different levels. Trauma within the lunula is more likely to cause irreparable nail changes than that of the nail bed [129]. This observation is true for adults and children alike, although the likelihood of normal regrowth is greater in children with similar trauma [130]. Longitudinal biopsies of the entire nail unit within the midzone of the nail are said to cause a chronic split if the width of the biopsy exceeds 3 mm [17]. However, there are several factors in addition to the width of the biopsy that can contribute to scarring with longitudinal biopsies and smaller biopsies in the midzone can also give long-term problems.

In some circumstances, most commonly old age, there is a pattern of subungual hyperkeratosis associated with nail thickening which gives the

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

impression of a nail bed contribution to the nail plate. Historically, this has been referred to as the solehorn and considered a germinal element of the hyponychium. Samman considered this issue in the context of a patient with pustular psoriasis [124]. He concluded that the ventral nail is a movable feast, manifesting itself in certain pathological circumstances.

Normal nail morphology

The main issues in normal nail morphology are: why is it flat? and why is the free edge rounded and not pointed? Factors influencing nail plate thickness are dealt with earlier (see 'Nail plate' above).

Why does nail grow out straight?

The first question was addressed in an article by Kligman [131] entitled, 'Why do nails grow out instead of up?'. His hypothesis was that the proximal nail fold acts to mould the nail as it moves away from the matrix giving an oblique growth path.

Baran [132] presented evidence to the contrary from surgical experience in the removal of the proximal nail fold and the lack of subsequent change in the nail. There are further observations concerning the relevance of acquired bone and nail changes occurring in tandem. Carpal tunnel syndrome can result in abnormal nails alongside acroosteolysis and ischaemic skin lesions [133, 134]. The reversal of many of the skin and nail features on treatment of the carpal tunnel compression suggests a neurovascular origin to both nail and bone changes in this pattern of acroosteolysis. Where the aetiology of the osteolysis is termed idiopathic, there are also nail changes [135]. It seems unlikely that these cases represent a specific influence of bone upon nail formation, but rather that both structures are responding to some undefined agent. There are a wide range of primary disorders in which secondary osteolysis and altered nails are recognized complications [135].

All the models demonstrating the influence of the different periungual tissues and bone upon the nail are flawed. Those above do not acknowledge the adherent quality of the nail bed as an influential factor, or the guiding influence of the lateral nail being embedded in the lateral nail folds.

What determines the contour of the free edge?

The second issue is why are nails rounded and not pointed? This has generally been accepted as being a function of the shape of the distal margin of the lunula, as illustrated in Fig. 23. Given that nails are growing continuously throughout life, it is possible to argue that we rarely see the true free edge, but observe the eroded or manicured outline. However, there are two instances when we see the genuine free edge; at birth and with regrowth following avulsion. These appear to follow the margin of the lunula. Finally, the nail bed may have some role in determining the shape of the free edge. Trauma to the nail bed can result in nail plate dystrophies giving the free edge a scalloped contour.

Nail growth measurement

A range of methods have been employed to measure nail growth, mostly requiring the imprint of a fixed reference mark on the nail and measuring its change in location relative to a fixed structure separate from the nail after a study period. Gilchrist and Dudley Buxton [136] made a transverse scratch about 2 mm from the most distal margin of the lunula. This distance was then measured using a rule and magnifier. Changes in the distance with time provided a record of growth rate. There have been variants of this, with the scratch being made at the convex apogee of the lunula and subsequent measurements made with reference to the lunula [137], or alternatively making a scratch a fixed 3 mm from the cuticle and noting the change with time [138]. The precision of these methods was increased by the introduction of magnified photographs before and after, and comparison of

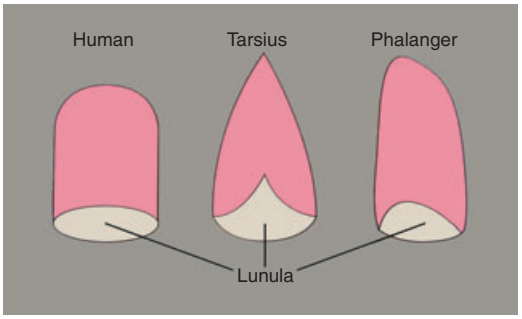


Figure 23 Relationship of lunula to nail tip shape.

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

the photographs [139]. This was modified further by Sibinga [140] who increased the photographic magnification from a factor of 6 to 35. This made it possible to conduct studies of nail growth over a period as short as 1 month.

Surface imaging of the nail, exploiting natural irregularities, can be used in lieu of marks placed by the observer. This has been reported by de Doncker and Piérard [141] in a study of nail growth during itraconazole therapy. The technique was not pushed to its full potential, as only clippings and not the entire *in vivo* nail plate were assessed. It was thought that longitudinal nail growth increased during therapy because surface beading became more spaced apart.

All these methods involve estimation of linear growth. As a measure of total matrix activity this could be misleading. Hamilton *et al.* sought to measure volume by the following equation [142]:

$$\text{Thickness (mm)} \times \text{breadth (mm)} \times \text{length grown per day} = \text{volume}$$

In some diseases, this may alter the impression of reduced nail growth – where a nail that is thickened has a low longitudinal growth rate, but is still producing the same mass of nail. This observation has been made in Yellow nail syndrome, where 'the nail that grows half as fast grows twice as thick' [143]. Onychomycosis may also be either predisposed to slow growing nails or a factor in their slow growth [144] and directly or indirectly, the antifungal drugs intraconazole and fluconazole may hasten longitudinal growth rate [145]. Johnson *et al.* also tried to measure volumetric growth with respect to linear growth [126].

Physiological factors and nail growth

Most studies concern fingernails. Their rate of growth can vary between 1.9 and 4.4 mm $month^{-1}$ [141]. A reasonable guide is 3 mm $month^{-1}$ or 0.1 mm day^{-1}. Toenails are estimated to grow around 1 mm $month^{-1}$. Population studies on nail growth have given the general findings that there is little marked seasonal change and nails are unaffected by mild intercurrent illnesses [137, 141]. The height or weight of the individual made no significant difference [137, 146]. Sex makes a small difference in early adulthood, with men having significantly ($P < 0.001$) faster linear nail growth up to the age of 19 [143]. They continue to do so with gradually diminishing significance levels, up to the age of 69, when there is a cross-over and women's nails grow faster than males'. There is rough agreement from Hillman in an earlier study, although he found that the crossover age was around 40 [137]. However, males continued to have a greater rate of nail growth throughout life if volume was measured, and not length [143]. Children under 14 have faster growth than adults.

Pregnancy may increase the rate of nail growth [146] and poor nutrition may retard it [136]. Temperature is an influence with unclear effects. Bean [147] kept a record of his own fingernail growth by making a scratch at the free edge of his cuticle on the first day of each month for 35 years. His record showed a gradual slowing with age. It initially showed a seasonal variation with heightened growth in the warm months. This variation became less marked with age, combining with a move from Iowa to Texas where seasonal contrasts are reduced. Other studies to determine the influence of temperature have compared nail growth rates for people in temperate and polar conditions. An original study in 1958 [148] found that nail growth was significantly retarded by living in the Arctic. Subsequent studies from the Antarctic found that there was no change in nail growth [149, 150]. These studies are not scientific, and it is unclear whether they are commenting on the improvement in thermal insulation since 1958 or nail physiology.

Nail growth in disease

Systemic disease

Insufficient numbers of seriously ill people have been followed as part of a larger study to give good statistical evidence concerning the influence of disease on nail growth. There is plenty of evidence from small numbers that some severe systemic upsets disturb nail formation. The observations of JS Beau in 1836 [151] detailed the development of transverse depressions upon the nails of people surviving typhoid. The form of nail growth interference represented by Beau's lines is seen in many conditions. Severe illness in the form of mumps has been noted to bring linear growth to a standstill [141]. Paradoxically, Sibinga [140] found that cadavers appeared to continue the growth of their nails in the

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

10 days post mortem during which they were assessed. The effect of death was less marked than mumps; something adults with mumps might agree with.

Local disease

Local diseases can influence nail growth. Dawber [138] noted that onycholysis was associated with increased nail growth. This was true whether it was related to psoriasis or idiopathic.

Trauma may also influence nail growth, where wrist fractures are the most common example. Details of nail and local hair growth have been recorded in instances of reflex sympathetic dystrophy where Beau's lines and hypertrichosis on the dorsum of wrist, arm and hand may coincide. It is not clear whether the nail changes represent increased or decreased growth in these circumstances. Immobility alone may result in a reduction in rate of nail growth and although this factor prevails after wrist fracture, reflex sympathetic dystrophy entails significant changes in blood supply that may have their own effects.

An additional factor in local inflammatory nail diseases is the immune status of the appendage [152]. There is some evidence that the nail shares some immunological properties with the hair follicle in representing a zone of relative immune privilege. This has largely been defined in immunohistochemical terms, demonstrating downregulation of HLA antigen groups A, B and C, with upregulation of HLA-G^+. Within the proximal nail matrix there is strong immunoreactivity for potent, locally generated immunosuppressants such as transforming growth factor-beta1, alpha-melanocyte stimulating hormone, insulin-like growth factor-1, and adrenocorticotropic hormone. There are few CD1a(+), CD4(+), or CD8(+), NK and mast cells. This supports a pattern of immunoreactivity consistent with a reduction of antigen presenting capacity, suggesting that the nail unit may be relatively protected from sensitization. But possibly at the cost of impaired management of microbial attack. The caution to these interpretations lies in the origin of the observations, where the tissue was obtained from accessory digits in just three infants. Hence the age and small number of the samples may not be representative of the adult nail. Analysis of peptides in the adult nail reveals human cathelicidin LL-37 is also found in adult human and porcine nail. This is fungicidal to Candida and may represent an element of natural resistance [153]. In an environment where immunological responses may be altered, this could be or particular importance.

Table III includes influences upon nail growth that are reported, but not always of statistical significance.

Nail plate biochemical analysis

Methods of analysis

A great range of methods have been used to analyse the organic and inorganic content of nails. Nail hydration is a central factor in the nature and quantities of other constituents. Near infrared spectroscopy can be used *in vivo* [154]. In 15 subjects it has demonstrated that the average water content of the nail plate is significantly lower in winter than in summer ($P < 0.05$). Nail water content is also reduced where there are splits in the nail.

Nail proteins and lipid

From Table IV on analytical methods it is clear that a considerable number of endogenous and exogenous materials can be sought in the nail plate. The protein mesh into which the elements fit is made primarily of the intracellular protein

Table III Influences in nail growth

Faster	Slower
Daytime	Night
Pregnancy	First day of life
Minor trauma/nail biting	
Right hand/dominant	Left hand/non-dominant
Youth	Old age
Fingers	Toes
Summer [147]	Winter/cold
Middle finger	Thumb and little finger
Men	Women
Psoriasis	Finger immobilization
Pitting	Fever
Normal nails	Beau's lines
Onycholysis	
Pityriasis rubra pilaris	Methotrexate, azathioprine
Etretinate	Etretinate
Idiopathic onycholysis of women	Denervation
	Poor nutrition
Hyperthyroidism	
l-DOPA	
AV shunts	Yellow nail syndrome
Calcium/vitamin D	Relapsing polychondritis
Benoxaprofen	

Source: Ref. [2].

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

Table IV Different methods of nail constituent analysis

Method	Element
Structural and mineral constituentsa	
Raman spectroscopy	Water, proteins and lipid [155]
Immunohistochemistry	Keratin [118]
Polymerase chain reaction	Deoxyribonucleic acid [180, 181]
Electron microscopy	Cystine
X-ray diffraction	Mg, Cl, Na, Ca, S, Cu [158, 168]
Colorimetry	Fe [170]
Exogenous materials	
Atomic absorption spectometry	Cd, Pb, Zn, Ca, Cr, Fe, Cu, Mn, Ni, Co, Na, K [158, 184]
Mass fragmentography	Metamphetamine [191]
Gas chromatography	Amphetamine, cocaine [190, 192, 193]
Flow injection hydride generation atomic absorption spectrometry	Arsenic [185]
Biological markers	
High-performance liquid chromatography	Furosine (glycosylated keratin), Terbinafine [172]
Microscopy	Lipid: triglyceride
Adsorption differential pulse voltametry	Ni [189]
Enzymic assay	Steroid sulphatase
Neutron activation analysis	Zinc, selenium [175, 176]

Source: Ref. [2].

keratin. The highly ordered structure of the proteins in the nail plate helps explain the degree of chemical and physical resistance in contrast to the characteristics of skin. The proteins of hair and nail alike have extensive folding maintained by extremely stable disulphide bonds. Although these bonds can also be found to a lesser extent in the stratum corneum of normal skin, they have a different geometry in the two sites as demonstrated by Raman spectroscopy. This is expressed as *gauche-gauche-gauche* for hair and nail and *gauche-gauche-trans* for stratum corneum [155]. The latter is less stable. The altered geometry of disulphide bonds and the extreme folding of protein molecules in hair and nail results in a different degree of hydration. The looser structure of skin allows more free water, whereas the structure of hair and nail allows very little. This contrasting degree of hydration means that skin is capable of sustaining metabolic processes not seen in nail [155]. Keratins and the associated proteins fall into the following categories:

- low sulphur proteins (40–60 kDa);
- high sulphur proteins (10–25 kDa);
- high glycine/tyrosine proteins (6–9 kDa).

It is believed that the low sulphur keratins form 10-nm filaments and the latter two groups of proteins form an interfilamentous matrix. The diversity of keratins within humans and between different species lies in the permutations of these three proteins [156] and the diversity of the keratins themselves. Over 30 high sulphur proteins have been identified in human nail by polyacrylamide gel electrophoresis [157].

Nail plate keratin fibrils appear orientated in a plane parallel to the surface and in the transverse axis [158]. They fall roughly into an 80:20 split between 'hard' hair type (trichocyte) keratin and 'soft', epithelial keratin [159]. These two variants are similar in many respects and share an X-ray diffraction pattern of α-helices in a coiled conformation, also confirmed using Raman spectroscopy [155]. Hard keratins split into the classical association of acidic and basic pairs, with extensive amino acid homologies with the epithelial forms [160]. In spite of regions of homology, the 'hard' and 'soft' keratins are distinguishable by immunohistochemistry [118, 159, 161]. The relative resilience of the two groups of keratins is also reflected in their solubility in 2-mercaptoethanol. At 50 mmol L^{-1} concentrations, only epithelial 'soft' keratins are extracted from nail clippings. The concentration needs to be raised to 200 mmol L^{-1} before significant quantities of 'hard' keratin dissolve [162]. Other cocktails for the extraction of proteins include mixes of thiourea and urea, which has been used in analysis of hair, feather and nail [163].

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

The main lipid of nail is cholesterol which increases with age [164]. However, the level of cholesterol sulphate decreases with age and this is most marked in women. It has been proposed that this might play some part in the tendency for nails to become more brittle with time. There is no clear relationship with serum lipids. The total fat content is 0.1–1%, contrasting with the 10% found in the stratum corneum. The water content is less than that of skin, being 7–12% compared with 15–25%. The lipid composition of nail varies with age and is more akin the composition of the sole of the foot than other areas of skin. There is a high proportion of free fatty acids and a low proportion of free sterols [165].

Mineral constituents of nail

X-ray diffraction is one of the best tried methods of elemental nail analysis. Much of the initial work was done by Forslind [158]. He observed that the hardness of the nail plate is unlikely to be due to calcium, which the analogy with bone has suggested. Detailed resumés of normal nail mineral content have been made [166].

Much interest has been demonstrated in the analysis of nails as a source of information concerning health. A significant increase in the nail content of Na, Mg and P was noted in a survey of 50 patients with cirrhosis [167]. In a comparison of term and preterm infants, a decrease in aluminium and sulphur was found in term deliveries. The high aluminium content in preterm infants was considered of possible relevance to the osteopenia observed in this group [168]. Copper and iron have been observed at higher levels in the nails of male 6- to 11-year olds in comparison with females [169]. Iron levels in the general population were found to be equal in men and women, but higher in children and highest in the neonate [170].

Biological markers in nail plate

In some respects, nail analysis can be compared with a blood test, but involving the examination of a less labile source of information. Analysis of chloride in nail clippings of a juvenile control population and those suffering cystic fibrosis, revealed a significant increase of chloride, by a factor of 5, in the latter. This has led to the suggestion of 'screening nail by mail' for inaccessible regions, where sending nails would be relatively easy.

The glycosylated globin molecule, used for estimation of long-term diabetic control, has been used as a model in studies measuring nail furosine in diabetes mellitus. The nail fructose-lysine content is raised in this disease and has shown a correlation with the severity of diabetic retinopathy and neuropathy [171]. Nail furosine levels have also shown a good correlation with fasting glucose and may even compete with glycosylated haemoglobin as an indicator of long-term diabetic control [172].

Nanoindentation is a technique for measuring the microscopic deformity of the surface of the nail under the influence of a defined focal force. It represents a measure of hardness, although the colloquial meaning of hard nails may cover a range of characteristics. It has been employed to attempt to determine if there is a correlation between osteoporosis and loss of nail hardness. A preliminary study demonstrated a statistically non-significant correlation [173].

Selenium is a trace element critical for the activity of glutathione peroxidase, which may protect DNA and other cellular molecules against oxidative damage. High concentrations are seen to protect against the action of certain carcinogens in some animal models and consequently its role in human cancers has been explored. Analysis of the selenium levels of different rat tissues suggest that blood selenium may be the best indirect measure of liver selenium and nail selenium may best reflect whole body levels and the level in skeletal and heart muscle [174]. Nail selenium levels in those being screened for oral cancer [175] and carcinoma of the breast [166, 176] showed no significant differences between affected and control patients. However, in a prospective study, toenail selenium levels had a weak predictive value for the development of advanced prostate cancer, where low levels of selenium predisposed to this malignancy [177]. Examination of a wide range of trace elements in the nails of women with breast cancer failed to show any difference from normal controls [178] and analysis of nail for zinc showed no significant difference between pellagra patients with low serum zinc and normal controls [179].

Nail clippings can be used as a source of DNA which, after amplification by the polymerase chain reaction, is of forensic use. Early work required 20–30 mg of nail [180] and this figure has decreased to 9 mg, where the DNA for the HLA-DQa alleles are used to assess homology with

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

blood samples [181]. More recently, 5 mg samples of nail have been analysed for both normal nuclear DNA and mitochondrial DNA [182]. Both are detectable with short tandem repeat profiling and mitochondrial DNA sequencing, respectively. As the nail sample gets older, only the latter remains definable and was of proven validity 8 years after cutting. The techniques are sufficiently precise as to allow differentiation of DNA deriving from the nail and that of possible contamination from material on the nail surface.

Hepatitis B viral DNA can also be identified in nail clippings using PCR, but there has not been sufficient work on this to determine if it is a reliable form of analysis [183].

Exogenous materials in nail analysis

Exogenous materials can be considered in two groups: environmental and ingested substances. In the first category, cadmium, copper, lead and zinc were examined in the hair and nails of young children [184]. This was done to gauge the exposure to these substances sustained in rural and industrialized areas of Germany. Both hair and nail reflected the different environments, although the multiple correlation coefficient was higher for hair than nails.

Water taken from wells in arsenic-rich rock has resulted in arsenic poisoning on a major scale in West Bengal, India over the last 20 years. About 50% of ingested arsenic is excreted in the urine, smaller amounts in the faeces, hair and nails. Nail analysis has been used in the Bengal population as well as in other populations suffering arsenic poisoning. Levels were estimated using flow injection hydride generation atomic absorption spectroscopy which allows analysis using very small samples and enables comparisons between different tissues. The Bengal experience suggests that there are similar concentrations in hair and nail, with a trend towards higher concentrations in the latter [185]. During an episode of arsenic poisoning in Alaska, the level of arsenic in nail was four times that found in hair [186]. A study in New Hampshire, U.S.A., found that in subjects drinking from arsenic-rich wells, there was a doubling of toenail arsenic for a 10-fold rise in water arsenic content [187].

Positive aspects of water content can also be monitored, such as the addition of fluoride in dental caries prevention projects. A direct correlation has been demonstrated between fluoride content of drinking water and that of nail in Brazilian children using high definition mass spectrometry [188].

Nickel analysis has been performed to establish occupational exposure [189]. The use of forensic nail drug analysis has been reported by the Japanese where over 20 000 people were arrested for the abuse of methamphetamine in 1987 [190, 191]. It was found that the drug entered the nail via both the matrix and nail bed. Chronic drug abusers could be distinguished from those with a single recent ingestion by scraping the undersurface of the nail before analysis. This would remove the nail bed contribution and the drug it contained in the 'one-off' abuser.

Simultaneous hair and nail analysis has been performed to compare the capacity of the tissues to reflect chronic drug abuse in those taking cocaine [192] and amphetamines [193]. Miller *et al.* [192] found that concentrations of cocaine and its derivatives were higher in hair than in nail, whereas Cirimele *et al.* [39] found that the concentrations of amphetamines and its metabolites were similar in both tissues. Analysis of nail clippings from the newborn by gas chromatography-mass spectroscopy can provide evidence of exposure to cocaine during embryogenesis.

The presence of therapeutic drugs has also been measured, and in particular, antifungal agents such as terbinafine and itraconazole [194]. There is considerable variation in the results of different studies looking at these drugs, but the common ground appears to be that the drug is incorporated into the nail plate via the nail bed as well as at the point of nail generation. This means that drug can be found in distal nail plate sooner than would be the case if it were necessary to wait for full nail growth. But the movement may be two ways as there is also evidence that the nail plate may then lose drug once treatment is stopped, so that a zone of nail plate which was created during drug treatment will contain no drug several months later.

Physical properties of nails

Strength

The strength and physical character of the nail plate is attributable to both its constituents and design. The features of design worthy of note are the double curvature, in transverse and longitud-

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

inal axes, and the flexibility of the ventral plate compared with the dorsal aspect. The first provides rigidity, whereas the latter allows moderate flexion deformity and slightly less extension. The most proximal component of the matrix provides the corneocytes of the dorsal nail surface. These usually provide a shiny surface. When the matrix is altered by disease or the nail surface subject to trauma, this shine is lost.

Measuring nail strength

Several techniques have been developed to study the physical properties of nails [173, 195–197]. Maloney's studies showed changes of tensile, flexural and tearing strength with age, sex and the digit from which the nail derived. Finlay devised a 'nail flexometer' able to repeatedly flex longitudinal nail sections through 90°, recording the number it took to fracture the nail. In this way, the strength could be quantified. He noted that the immersion of nails in water for an hour increased their weight by 21%. It also made them significantly more flexible. After 2 h, the flexibility was still increasing, although the water content reached a plateau. Analysis of *in vivo* nail by Raman spectroscopy suggests that after soaking in water for 10 min, the α-helical protein conformation is made more loose, with greater spacing between proteins as water occupies the interstices. However, this change is seen only in distal nail, with proximal nail already manifesting a high degree of hydration before immersion [198].

Mineral oil has no effect on flexibility, although it can act to maintain some of the flexibility imbued by water. This principle is applied in the treatment of onychoschizia, where repeated hydration and drying of the nail plate results in splitting at the free edge [95]. Zaun [199] used a method of assessment of brittleness that relies on the swelling properties of nail, employing the technique before and after therapy for brittle nails. Splitting can be partially overcome by applications of emollient after soaking the nails in water. The use of nail varnish can also decrease water loss [200].

Shearing and cutting studies on clipped nail samples indicate that the amount of force needed to cut a nail is three times greater in the longitudinal axis than it is in the transverse axis. It is argued that this is due to the main axis and shape of the corneocytes in the predominant intermediate zone of the nail plate [201].

Permeability

The degree of nail plate swelling in alkali has been suggested as an index of disease [202]. Normal, onychomycotic and psoriatic nails differ in the time it takes them to stop swelling, and what percentage volume increase they sustain.

Nail permeability is relevant to topical drugs on the dorsal surface and systemic drugs from the ventral surface. Transonychial water loss can be measured *in vivo* [203], but drug penetration assay is more complicated. The simplest method is to use cadaver nails. Doing this, the permeability coefficient for water has been estimated at 16.5×10^{-3} cm h^{-1} and that for ethanol at 5.8×10^{-3} cm h^{-1} [204]. This demonstrates that the hydrated nail is more permeable to water than to alcohol and behaves like a hydrogel of high ionic strength to polar and semipolar alcohols. Combining alcohols with water may increase the permeation by the alcohol. The addition of *N*-acetyl-L-cysteine or mercaptoethanol to an aqueous solvent has been found to enhance the penetration of nail samples by the antifungal, tolnaftate [205]. Human nail can be substituted in such studies using an *in vitro* model for the assessment of drug penetration employing a keratin membrane prepared from bovine [206], sheep [207] and porcine hooves [208].

The nail is 1000 times more permeable to water than is skin [200, 209]. One means of assessing this is using an evaporimeter to measure transonychial water loss [210]. When this is done in healthy and diseased nails, it appears that healthy nail is significantly more permeable. This could be attributable to the nail plate or the relationship between the nail plate and nail bed. In diseases such as psoriasis and onychomycosis, there is less intimate apposition between nail and nail bed.

The ease with which water passes through nail means that drugs required to diffuse through the nail should normally have a high degree of water solubility [211, 212]. In spite of this, there is possibly a parallel lipid pathway that allows permeation of hydrophobic molecules [213] and lipid vehicles are of value because they stick better to the nail surface [206, 212].

Molecular size, expressed as weight, is a further factor determining the penetration of nail by a drug. Larger molecules penetrate less well. In the field of topical antimycotics, this allows prediction

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

of efficacy when the combined characteristics of water solubility, molecular size and minimum inhibitory concentration for antifungal activity are allowed for in a complex calculation [214]. In this manner, drugs such as ciclopirox and amorolfine can be predicted to be of some value. However, more hydrophobic molecules, such as the imidazoles, itraconazole and ketoconazole, are barely able to diffuse into nail, even when it is pretreated with topical keratolytics such as papain, urea and salicylic acid [215].

The dense matrix of keratin and associated proteins is considered an obstacle to dimethylsulphamethoxysole (DMSO) penetration in the nail plate, contrasting with its easier access through skin [213]. However, it appears to facilitate the penetration of some topical antimycotics [216]. When amorolfine is applied to nail, its penetration is enhanced by pretreatment with DMSO and the penetration is further enhanced if methylene chloride is used as a vehicle for the antifungal in preference to ethanol [217]. Some medicated lacquers are also able to penetrate sufficiently to be of clinical use, particularly if their access is increased by abrading the dorsal surface of the nail plate [218, 219]. The concentration gradient, and hence diffusion, can be increased further by facilitating solution of the reagents, such as miconazole, by lowering the pH [220]. Using a solvent that evaporates will have the same effect [221]. Other facilitating vehicles include *N*-(2-mercaptopropionyl) glycine, a mercaptan derivative of an amino acid, in combination with urea [222]. One factor in nail plate penetration will be the persistence of the drug and its vehicle on the dorsal aspect of the nail after application. Studies comparing residues of nail lacquer antifungal products illustrate that there can be substantial differences between products in this respect [223].

There is also exchange of chemicals between nail and the internal environment and it is likely that the nail has different characteristics of drug penetration on the dorsal and ventral surfaces [224]. The significance of this is mainly with respect to inclusion of circulating materials into the nail rather than the other way around, although in a study of topical application of sodium pyrithone, Mayer *et al.* [225] found microscopic amounts in the systemic circulation. Munro and Shuster [226] and Matthieu *et al.* [227] have shown that drugs can penetrate rapidly into the distal nail via the nail bed. Other drugs may be found in the nail, which makes the nail a useful source of information concerning the ingestion of some illicit drugs or environmental pollutants (see below).

Radiation penetration

Chronic X-irradiation is associated with carcinoma *in-situ* and invasive squamous cell carcinoma [228]. The polydactylous form of Bowen's disease is commonly related to some source of radiation [229]. Parker and Diffey [230] have investigated the transmission of light through the toenails of cadavers. Examining wavelengths between 300 and 600 nm, it appears that transmission at the shorter wavelength is minimal. This corresponds to UVB. If the nail plate is acting as a sunscreen it is fortuitous, but the character of the toenails studied may not be the same as fingernails, which are more commonly exposed.

A double-blind study of superficial radiotherapy in psoriatic nail dystrophy has demonstrated a definite albeit temporary benefit [231]. A similar temporary benefit has been demonstrated with electron beam therapy [232]. Both of these studies might suggest that the different forms of radiation are penetrating nail, although treatment of periungual psoriasis can have a secondary beneficial effect on subungual tissues.

Imaging of the nail apparatus

Radiology

X-ray reveals little of the soft structures of the nail unit under normal circumstances. Glomus tumours may provide particular radiological features. Mathis and Schulz [233] reviewed 15 such tumours on the digit and found that nine had characteristic changes of bony erosion. This was smooth and concave in most cases, but occasionally with a punched out appearance on the phalangeal tuft. Supplementation of routine X-rays with arteriography may reveal a star-shaped telangiectatic zone [234].

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is an effective method of locating tumours, particularly where there is diagnostic difficulty [235–237]. It can be used in a range of periungual neoplasms [238], although it is most useful when the tumour contrasts with surrounding tissues with respect to

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

density, fluid or fat content. The most marked example of this is with mucoid cysts but even normal soft tissues can be differentiated and an *in vivo* anatomical assessment made using MRI, characterizing normal matrix and submatrix anatomy [21]. It has also been used to establish the coincidence between nail dystrophy and joint changes in psoriatic arthritis [239].

Ultrasound

Ultrasound has been used in the nail unit both as a research tool and to aid clinical diagnosis. Finlay *et al.* [84] and Wollina *et al.* [240] have used a 20-MHz pulse echo ultrasound to measure nail thickness *in vivo*, proximally and distally. Finlay's work implies that the nail becomes thinner as it emerges, which is contrary to findings on avulsed nails (see 'Nail growth' above). He also found that the nails ranked in thickness sequentially around the hand, with the thumb being top and the little finger bottom. Wollina looked at normal controls and those with disease. In some diseases, such as systemic sclerosis and systemic lupus erythematosus, there were changes in matrix volume and nail plate.

Jemec's study of cadaver nails, *in situ* and avulsed, showed that nail dessication destroyed the correlation between ultrasound thickness measurements and screw gauge [241]. This could have significance in quantification when the water content of nails can vary by 10%.

Clinically, high frequency ultrasound has been used in the diagnosis of glomus tumours. Fornage examined 12 patients and could depict the tumour in nine. The resolution of his transducer meant that lesions smaller than 3 mm could not be seen [242]. Hirai and Fumiiri [243] have used a 30-MHz high resolution B-mode ultrasound probe to examine nail dystrophies. The conclusion was that if the echogram detected any abnormality beneath the nail fold, the dystrophy was likely to be due to matrix pathology. The converse was true for nail bed pathology. The authors claimed that even in poorly defined tumours such as subungual malignant melanoma, the technique provided guidance as to the tumour margin.

Profilometry

Profilometry is the technique of measuring the profile of a surface. This has been used in skin for nearly 20 years and can give a measure of wrinkling with actinic damage [244, 245]. More recently it has been used on nail surfaces to assess dystrophies where characteristic profiles are reported for the clinical features of pitting, grooves and trachyonychia [246]. Having established the method, it is then possible to use it as a measure of disease activity and nail growth as attempted with a study of psoriatic trachyonychia during low-dose cyclosporin therapy [247] and the rate of nail growth during itraconazole treatment [141].

Dermoscopy of nail

Nail dermoscopy is a method of microscopic examination using reflected light [103]. In the clinical setting this is conveniently provided by a hand-held dermatoscope. A dissection microscope is a laboratory alternative. The dermatoscope affords only low magnification, usually in the order of 10–20×. Illumination is provided from a light source within the instrument with an incident angle of 20°. LEDs are the brightest and most effective. Electrode or ultrasound gel is used on the nail surface to remove the air interface and reflection, so enhancing depth of nail plate examination. This technique is of particular value when examining pigmented changes of the nail unit and differentiating subungual blood from melanin within the nail plate [248].

Confocal microscopy

Confocal microscopy has been used in a small number of studies to examine near-surface phenomena in nail [249]. This has particularly been applicable to onychomycosis and can be undertaken *in vivo* [250].

Light microscopy and electron microscopy

Light microscopy and scanning and transmission electron microscopy have been used to elucidate the normal ultrastructure of the nail (see 'Nail histology').

Photography

Many sophisticated systems are available for taking good photographs of the nail unit. Colour balance, even depth of focus and avoiding reflective glare from the nail plate surface are the main challenges. A ring flash overcomes some of these challenges, but the even illumination means that some of the visual cues of curvature and depth are

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

lost. Colour balance is usually a matter of adjusting the settings in the camera. This in turn will be influenced by the choice of background. As a general rule, lighter backgrounds suit digital cameras where exposure of the digit and nail will be influenced by the perceived ambient brightness.

Video systems have been used as a research tool for nail morphometry [251]. End-on and lateral views can be transferred to a computer for calculation of dimensions and provide reference measurements.

Other techniques

Laser Doppler can be used to assess the blood flow in the nail unit. Optical coherence tomography produces a series of cross-sectional images down to a depth of 1 mm, separated by 15 mm. It has some potential for examining periungual tissues, but has been little explored as a technique [252].

Older techniques for the assessment of shape, and clubbing in particular, include brass templates [253], shadowgraphs [254], plaster casts and planimetry [255] and plethysmography [256].

Acknowledgements

This study was funded by the National Disease Centre.

References

1. Lewis, B.L. Microscopic studies of fetal and mature nail and surrounding soft tissue. *Arch. Derm. Syphilol.* **70**, 732–744 (1954).
2. Dawber, R.P.R., Baran, R. and de Berker, D. The science of the nail apparatus. In: *Diseases of the Nail and Their Management*, Chapter 1, 3rd edn (Baran, R., Dawber, R., de Berker, D.A.R., Haneke, E. and Tosti, A., eds), p. 34. Blackwell Scientific Publications, Oxford (2001). ISBN 0-632-05358-5.
3. Zook, E.G. Anatomy and physiology of the perionychium. *Hand Clin.* **18**, 553–559 (2002).
4. Shum, C., Bruno, R.J., Ristic, S., Rosenwasser, M.P. and Strauch, R.J. Examination of the anatomic relationship of the proximal germinal nail matrix to the extensor tendon insertion. *J. Hand Surg. [Am.]* **25**, 1114–1117 (2000).
5. Reardon, C.M., McArthur, P.A., Survana, S.K. and Brotherston, T.M. The surface anatomy of the germinal matrix of the nail bed in the finger. *J. Hand Surg. [Br.]* **24**, 531–533 (1999).
6. Cabral, A., Berger, T.H., Middag-Broekman, J.H. and Boon, M.E. Unequivocal morphological diagnosis of fungi in morphologically abnormal nails. *Histopathology* **48**, 862–867 (2006).
7. Alkiewicz, J. and Pfister, R. *Atlas der Nagelkrankheiten*, p. 8. Schattauer-Verlag, Stuttgart. (1976)
8. Suarez, S.M., Silvers, D.N. and Scher, R.K. Histologic evaluation of nail clippings for diagnosing onychomycosis. *Arch. Dermatol.* **127**, 1517–1519 (1991).
9. Alvarez, R. and Zaias, N. A modified polyethylene glycolpyroxylin embedding method specially suited for nails. *J. Invest. Dermatol.* **49**, 409–410 (1967).
10. Bennett, J. Technique of biopsy of nails. *Dermatol. Surg.* **2**, 325–326 (1976).
11. Lewin, K., Dewitt, S. and Lawson, R. Softening techniques for nail biopsy. *Arch. Dermatol.* **107**, 223–224 (1973).
12. Haneke, E. Fungal infections of the nail. *Semin. Dermatol.* **10**, 41–53 (1991).
13. Achten, G. L'ongle normal et pathologique. *Dermatologica* **126**, 229–245 (1963).
14. Achten, G., Andre, J. and Laporte, M. Nails in light and electron microscopy. *Semin. Dermatol.* **10**, 54–64 (1991).
15. Achten, G. and Wanet, J. (1973) Pathologie der nagel. In: *Spezielle Pathologische Anatomie*, Vol. 7 (Doerr, W., Seifert, G. and Uehlinger, E., eds), pp. 487–528. Springer-Verlag, New York.
16. Baran, R. and Haneke, E. Diagnostik und Therapie der streifenformigen nagelpigmentierung. *Hautarzt* **35**, 359–365 (1984).
17. Zaias, N. The longitudinal nail biopsy. *J. Invest. Dermatol.* **49**, 406–408 (1967).
18. Nakayama, Y., Iino, T., Uchida, A. *et al.* Vascularised free nail grafts nourished by arterial inflow from the venous system. *Plast. Reconstr. Surg.* **85**, 239–245 (1990).
19. Pessa, J.E., Tsai, T.M., Li, Y. and Kleinert, H.E. The repair of nail deformities with the non-vascularised nail bed graft. Indications and results. *J. Hand Surg.* **15A**, 466–470 (1990).
20. de Berker, D.A.R., MaWhinney, B. and Sviland, L. Quantification of regional matrix nail production. *Br. J. Dermatol.* **134**, 1083–1086 (1996).
21. Drapé, J.L., Wolfram-Gabel, R., Idy-Peretti, I. *et al.* The lunula: a magnetic resonance imaging approach to the subnail matrix area. *J. Invest. Dermatol.* **106**, 1081–1085 (1996).
22. Wilkerson, M.G. and Wilkin, J.K. Red lunulae revisited: a clinical and histopathologic examination. *J. Am. Acad. Dermatol.* **20**, 453–457 (1989).
23. Cohen, P.R. The lunula. *J. Am. Acad. Dermatol.* **34**, 943–953 (1996).
24. Guero, S., Guichard, S. and Fraitag, S.R. Ligamentary structure at the base of the nail. *Surg. Radiol. Anat.* **16**, 47–52 (1994).

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

25. de Berker, D.A.R. and Baran, R. Acquired malalignment: a complication of lateral longitudinal biopsy. *Acta Derm. Venereol.* **78**, 468–470 (1998).
26. Dahdah, M.J. and Scher, R.K. Nail diseases related to nail cosmetics. *Dermatol. Clin.* **24**, 233–239 (2006).
27. Higashi, N. Melanocytes of nail matrix and nail pigmentation. *Arch. Dermatol.* **97**, 570–574 (1968).
28. Higashi, N. and Saito, T. Horizontal distribution of the DOPA-positive melanocytes in the nail matrix. *J. Invest. Dermatol.* **53**, 163–165 (1969).
29. Tosti, A., Cameli, N., Piraccini, B.M. *et al.* Characterization of nail matrix melanocytes with anti-PEP1, anti-PEP8, TMH-1, and HMB-45 antibodies. *J. Am. Acad. Dermatol.* **31**, 193–196 (1994).
30. de Berker, D., Dawber, R.P.R., Thody, A. and Graham, A. Melanocytes are absent from normal nail bed; the basis of a clinical dictum. *Br. J. Dermatol.* **134**, 564 (1996).
31. Perrin, C., Michiels, J.F., Pisani, A. and Ortonne, J.P. Anatomic distribution of melanocytes in normal nail unit: an immunohistochemical investigation. *Am. J. Dermatopathol.* **19**, 462–467 (1997).
32. Baran, R. and Kechijian, P. Longitudinal melanonychia. Diagnosis and management. *J. Am. Acad. Dermatol.* **21**, 1165–1175 (1989).
33. Hashimoto, K. Ultrastructure of the human toenail. II. *J. Ultrastruct. Res.* **36**, 391–410 (1971).
34. Zaias, N. Embryology of the human nail. *Arch. Dermatol.* **87**, 37–53 (1963).
35. Monash, S. Normal pigmentation in the nails of negroes. *Arch. Dermatol.* **25**, 876–881 (1932).
36. Leyden, J.J., Spot, D.A. and Goldsmith, H. Diffuse banded melanin pigmentation in nails. *Arch. Dermatol.* **105**, 548–550 (1972).
37. Kopf, A.W. and Waldo, F. Melanonychia striata. *Australas. J. Dermatol.* **21**, 70 (1980).
38. Oropeza, R. Melanomas of special sites. In: *Cancer of the Skin: Biology, Diagnosis, Management*, Vol. 2. pp. 974–987. Blackwell Scientific Publications, Oxford (1986).
39. Hashimoto, K. The marginal band: a demonstration of the thickened cellular envelope of the human nail. *Arch. Dermatol.* **103**, 387–393 (1971).
40. Hashimoto, K. Ultrastructure of the human toenail: I proximal nail matrix. *J. Invest. Dermatol.* **56**, 235–246 (1971).
41. Hashimoto, K. Ultrastructure of the human toenail. Cell migration, keratinization and formation of the intercellular cement. *Arch. Dermatol. Forsch.* **240**, 1–22 (1971).
42. Parent, D., Achten, G. and Stouffs-Vanhoof, F. Ultrastructure of the normal human nail. *Am. J. Dermatopathol.* **7**, 529–535 (1985).
43. Allen, A.K., Ellis, J. and Rivett, D.E. The presence of glycoproteins in the cell membrane complex of variety of keratin fibres. *Biochim. Biophys. Acta* **1074**, 331–333 (1991).
44. Zook, E.G. Anatomy and physiology of the perionychium. *Hand Clin.* **18**, 553–559 (2002).
45. Lewin, K. The normal finger nail. *Br. J. Dermatol.* **77**, 421–430 (1965).
46. Maricq, H.R. Observation and photography of sweat ducts of the fingers in vivo. *J. Invest. Dermatol.* **48**, 399–401 (1967).
47. Rayan, G.M. and Flournoy, D.J. Microbiologic flora of human fingernails. *J. Hand Surg.* **12A**, 605–607 (1987).
48. Arrowsmith, V.A., Maunder, J.A., Sargent, R.J. and Taylor, R. Removal of nail polish and finger rings to prevent surgical infection. *Cochrane Database Syst. Rev.* **4**, CD003325 (2001).
49. Dowsett, S.A., Archila, L., Segreto, V.A. *et al.* *Helicobacter pylori* infection in indigenous families of Central America: serostatus and oral and fingernail carriage. *J. Clin. Microbiol.* **37**, 2456–2460 (1999).
50. Lynch, M.H., O'Guin, W.M., Hardy, C. *et al.* Acid and basic hair/nail ('hard') keratins: their colocalisation in upper corticle and cuticle cells of the human hair follicle and their relationship to 'soft' keratins. *J. Cell Biol.* **103**, 2593–2606 (1986).
51. Heid, W.H., Moll, I. and Franke, W.W. Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. II. Concomitant and mutually exclusive synthesis of trichocytic and epithelial cytokeratins in diverse human and bovine tissues. *Differentiation* **37**, 215–230 (1988).
52. Moll, I., Heid, H.W., Franke, W.W. and Moll, R. Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. *Differentiation* **39**, 167–184 (1988).
53. Lacour, J.P., Dubois, D., Pisani, A. and Ortonne, J.P. Anatomical mapping of Merkel cells in normal human adult epidermis. *Br. J. Dermatol.* **125**, 535–542 (1991).
54. Sinclair, R.D., Wojnarowska, F. and Dawber, R.P.R. The basement membrane zone of the nail. *Br. J. Dermatol.* **131**, 499–505 (1994).
55. de Berker, D., Wojnarowska, F., Sviland, L. *et al.* Keratin expression in the normal nail unit: markers of regional differentiation. *Br. J. Dermatol.* **142**, 89–96 (2000).
56. Perrin, C, Langbein, L. and Schweizer, J. Expression of hair keratins in the adult nail unit: an immunohistochemical analysis of the onychogenesis in the proximal nail fold, matrix and nail bed. *Br. J. Dermatol.* **151**, 362–371 (2004).
57. Purkis, P.E., Steel, J.B., Mackenzie, I.C. *et al.* Antibody markers of basal cells in complex epithelia. *J. Cell. Sci.* **97**, 39–50 (1990).

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

58. de Berker, D., Sviland, L. and Angus, B.A. Suprabasal keratin expression in the nail bed: a marker of dystrophic nail differentiation. *Br. J. Dermatol.* **133**(Suppl. 45), 16 (1995).
59. Fanti, P.A., Tosti, A., Cameli, N. and Varotti, C. Nail matrix hypergranulosis. *Am. J. Dermatopathol.* **16**, 607–610 (1994).
60. Westgate, G.E., Tidman, N., de Berker, D. *et al.* Characterization of LH Tric-1, a new monospecific monoclonal antibody to the trichocyte keratin Ha1. *Br. J. Dermatol.* **137**, 24–30 (1997).
61. Okazaki, M., Yoshimura, K., Fujiwara, H., Suzuki, Y. and Harii, K. Induction of hard keratin expression in non-nail-matrical keratinocytes by nail-matrical fibroblasts through epithelial–mesenchymal interactions. *Plast. Reconstr. Surg.* **111**, 286–290 (2003).
62. Kitahara, T. and Ogawa, H. Coexpression of keratins characteristic of skin and nail differentiation in nail cells. *J. Invest. Dermatol.* **100**, 171–175 (1993).
63. Kitahara, T. and Ogawa, H. Variation of differentiation in nail and bovine hoof cells. *J. Invest. Dermatol.* **102**, 725–729 (1994).
64. Kitahara, T. and Ogawa, H. Cellular features of differentiation in the nail. *Microsc. Res. Tech.* **38**, 436–442 (1997).
65. Picardo, M., Tosti, A., Marchese, C. *et al.* Characterisation of cultured nail matrix cells. *J. Am. Acad. Dermatol.* **30**, 434–440 (1994).
66. Nagae, H., Nakanishi, H., Urano, Y. and Arase, S. Serial cultivation of human nail matrix cells under serum-free conditions. *J. Dermatol.* **22**, 560–566 (1995).
67. Boot, P.M., Rowden, G. and Walsh, N. The distribution of Merkel cells in human fetal and adult skin. *Am. J. Dermatopathol.* **14**, 391–396 (1992).
68. Munro, C.S., Carter, S., Bryce, S. *et al.* A gene for pachyonychia congenita is closely linked with the gene cluster on 17q12-q21. *J. Med. Genet.* **31**, 675–678 (1994).
69. McLean, W.H.I., Rugg, E.L., Luny, D.P. *et al.* Keratin 16 and 17 mutations cause pachyonychia congenita. *Nat. Genet.* **9**, 273–278 (1995).
70. Corden, L.D. and McLean, W.H. Human keratin disease: hereditary fragility of specific epithelial tissues. *Exp. Dermatol.* **5**, 297–307 (1996).
71. Hohl, D. Steatocystoma multiplex and oligosymptomatic pachyonychia congenita of the Jackson-Lawler type. *Dermatology* **195**, 86–88 (1997).
72. Covello, S.P., Smith, F.J.D., Sillevis Smitt, J.H. *et al.* Keratin 17 mutations cause either steatocystoma multiplex or pachyonychia congenita type 2. *Br. J. Dermatol.* **17** 475–480 (1998).
73. Smith, F.J., Jonkman, M.F., van Goor, H. *et al.* A mutation in human keratin K6b produces a phenocopy of the K17 disorder pachyonychia congenita type 2. *Hum. Mol. Genet.* **7**, 1143–1148 (1998).
74. Bowden, P.E., Haley, J.L., Kansky, A. *et al.* Mutation of a type II keratin gene (K6a) in pachyonychia congenita. *Nat. Genet.* **10**, 363–365 (1995).
75. Swensson, O., Langbein, L., McMillan, J.R. *et al.* Specialised keratin expression pattern in human ridged skin as an adaptation to high physical stress. *Br. J. Dermatol.* **139**, 767–775 (1998).
76. Haneke, E. The human nail matrix a flow cytometric and immunohistochemical studies. In: *Clinical Dermatology in the Year 2000*, Book of Abstracts, May (1990).
77. Baden, H. Common transglutaminase substrates shared by hair, epidermis and nail and their function. *J. Dermatol. Sci.* **7**(Suppl.), S20–S26 (1994).
78. Manabe, M. and O'Guin, W.M. Existence of trichohyalinkeratohyalin hybrid granules: co-localisation of 2 major intermediate filament-associated proteins in non-follicular epithelia. *Differentiation* **58**, 65–75 (1994).
79. O'Keefe, E.J., Hamilton, E.H., Lee, S.C. and Steiner, P. Trichohyalin: a structural protein of hair, tongue, nail and epidermis. *J. Invest. Dermatol.* **101**, 65s–71s (1993).
80. Lavker, R.M., Risse, B., Brown, H. *et al.* Localisation of plasminogen activator inhibitor Type 2 (PAI-2) in hair and nail: implications for terminal differentiation. *J. Invest. Dermatol.* **110**, 917–922 (1998).
81. Diaz, A.A., Boehm, A.F. and Rowe, W.F. Comparison of fingernail ridge patterns of monozygotic twins. *J. Forensic Sci. CA* **35**, 97–102 (1990).
82. Apolinar, E. and Rowe, W.F. Examination of human fingernail ridges by means of polarized light. *J. Forensic Sci. CA* **25**, 154–161 (1980).
83. Johnson, M., Comaish, J.S. and Shuster, S. Nail is produced by the normal nail bed: a controversy resolved. *Br. J. Dermatol.* **125**, 27–29 (1991).
84. Finlay, A.Y., Moseley, H. and Duggan, T.C. Ultrasound transmission time: an in vivo guide to nail thickness. *Br. J. Dermatol.* **117**, 765–770 (1987).
85. Samman, P.D. and White, W.F. The 'yellow nail' syndrome. *Br. J. Dermatol.* **76**, 153–157 (1964).
86. Jarrett, A. and Spearman, R.I.C. The histochemistry of the human nail. *Arch. Dermatol.* **94**, 652–657 (1966).
87. Garson, J.C., Baltenneck, F., Leroy, F., Riekel, C. and Muller, M. Histological structure of human nail as studied by synchrotron X-ray microdiffraction. *Cell. Mol. Biol. (Noisy-le-grand)* **46**, 1025–1034 (2000).
88. Samman, P.D. The ventral nail. *Arch. Dermatol.* **84**, 1030–1033 (1961).

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

89. Jemec, G.B.E. and Serup, J. Ultrasound structure of the human nail plate. *Arch. Dermatol.* **125**, 643–646 (1989).
90. Germann, H., Barran, W. and Plewig, G. Morphology of corneocytes from human nail plates. *J. Invest. Dermatol.* **74**, 115–118 (1980).
91. Sonnex, T.S., Griffiths, W.A.D. and Nicol, W.J. The nature and significance of the transverse white band of human nails. *Semin. Dermatol.* **10**, 12–16 (1991).
92. Forslind, B. and Thyresson, N. On the structures of the normal nail. *Arch. Dermatol. Forsch.* **251**, 199–204 (1975).
93. Dawber, R.P.R. The ultrastructure and growth of human nails. *Arch. Dermatol. Res.* **269**, 197–204 (1980).
94. Shelley, W.B. and Shelley, D. Onychschizia: scanning electron microscopy. *J. Am. Acad. Dermatol.* **10**, 623–627 (1984).
95. Wallis, M.S., Bowen, W.R. and Guin, J.D. Pathogenesis of onychoschizia (lamellar dystrophy). *J. Am. Acad. Dermatol.* **24**, 44–48 (1991).
96. Scherer, W.P. and Scherer, M.D. Scanning electron microscope imaging of onychomycosis. *J. Am. Podiatr. Med. Assoc.* **94**, 356–362 (2004).
97. Liu, J. and Lei, P. Histopathologic and scanning electron microscope examination of the nail and hair in chronic mucocutaneous candidiasis. *J. Am. Acad. Dermatol.* **49**(2 Suppl. Case Reports), S154–S156 (2003).
98. Sangiorgi, S., Manelli, A., Congiu, T., Bini, A., Pilato, G., Reguzzoni, M. and Raspanti, M. Microvascularization of the human digit as studied by corrosion casting. *J. Anat.* **204**, 123–131 (2004).
99. Parent, D., Achten, G. and Stouffs-Vamhoof, F. Ultrastructure of the normal human nail. *Am. J. Dermatopathol.* **7**, 529–535 (1985).
100. Hashimoto, K. Ultrastructure of the human toenail. II. *J. Ultrastruct. Res.* **36**, 391–410 (1971).
101. Caputo, R., Gasparini, G. and Contini, D. A freeze-fracture study of the human nail plate. *Arch. Dermatol. Res.* **272**, 117–125 (1982).
102. Arnn, Y. and Stoehelin, L.A. The structure and function of spot desmosomes. *Int. J. Dermatol.* **20**, 331–339 (1981).
103. Hirata, S.H, Yamada, S., Almeida, F.A., Enokihara, M.Y., Rosa, I.P., Enokihara, M.M. and Michalany, N.S. Dermoscopic examination of the nail bed and matrix. *Int. J. Dermatol.* **45**, 28–30 (2006).
104. Gilje, O., Kierland, R. and Baldes, E.J. Capillary microscopy in the diagnosis of dermatologic diseases. *J. Invest. Dermatol.* **22**, 199–206 (1974).
105. Ryan, T.J. Direct observations of blood vessels in the superficial vasculature system of the skin. In: *The Physiology and Pathophysiology of the Skin*, Vol. 2 (Jarrett, A., ed.), pp. 658–659. Academic Press, London (1973).
106. Davies, E. and Landau, J. (eds) *Clinical Capillary Microscopy*. C.C. Thomas, Springfield, IL (1966).
107. Bollinger, A., Jäger, K., Roten, A., Timeus, C. and Mahler, F. Diffusion, pericapillary distribution and clearance of Na-fluorescein in the human nail fold. *Pflügers Arch.* **382**, 137–143 (1979).
108. Grassi, W., Felder, M., Thüring-Vollenweider, U. and Bollinger, A. Microvascular dynamics at the nail fold in rheumatoid arthritis. *Clin. Exp. Rheumatol.* **7**, 47–53 (1989).
109. Halfoun, V.L., Pires, M.L., Fernandes, T.J., Victer, F., Rodrigues, K.K. and Tavares, R. Videocapillaroscopy and Diabetes mellitus: area of transverse segment in nailfold capillar loops reflects vascular reactivity. *Diabetes Res. Clin. Pract.* **61**, 155–160 (2003).
110. Hafner, H.M., Thomma, S.R., Eichner, M., Steins, A. and Junger, M. The influence of Emla cream on cutaneous microcirculation. *Clin. Hemorheol. Microcirc.* **28**, 121–128 (2003).
111. Wilgis, E.F.S. and Maxwell, G.P. Distal digital nerve graft. *J. Hand Surg.* **4**, 439–443 (1979).
112. Zook, E.G. Injuries of the fingernail. In: *Operative Hand Surgery* (Green, D.P., ed.), 2nd edn. Churchill Livingstone, New York (1988).
113. Winkelmann, R.K. Similarities in cutaneous nerve end-organs. In: *Advances in Biology of Skin: Cutaneous Innervation* (Montagna, W., ed.), pp. 48–62. Pergamon, New York (1960).
114. Jansen, C.W., Patterson, R. and Viegas, S.F. Effects of fingernail length on finger and hand performance. *J. Hand Ther.* **13**, 211–217 (2000).
115. Achten, G. Normale histologie und histochemie des nagels. In: *Handbuch der Haut- und Geschlechtskrankeiten*, Vol. 1 (Jadassohn, J., ed.), pp. 339–376. Springer-Verlag, Berlin (1968).
116. Manabe, M. and O'Guin, W.M. Existence of trichohyalinkeratohyalin hybrid granules: co-localisation of 2 major intermediate filament-associated proteins in non-follicular epithelia. *Differentiation* **58**, 65–75 (1994).
117. Dekio, S. and Jidoi, J. Comparison of human hair and nail low-sulfur protein compositions on two-dimensional electrophoresis. *J. Dermatol.* **16**, 284–288 (1989).
118. Heid, H.W., Moll, I. and Franke, W.W. Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. *Differentiation* **37**, 215–230 (1988).
119. Ogden, G.R., Chisholm, D.M., Leigh, I.M. and Lane, E.B. Cytokeratin profiles in dyskeratosis congenita: an immunocytochemical investigation of lingual hyperkeratosis. *J. Oral. Pathol. Med.* **21**, 353–357 (1992).
120. Lewis, B.L. Microscopic studies of fetal and mature nail and the surrounding soft tissue. *Arch. Dermatol. Syph.* **70**, 732–744 (1954).

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

121. Zaias, N. and Alvarez, J. The formation of the primate nail plate. An autoradiographic study in the squirrel monkey. *J. Invest. Dermatol.* **51**, 120–136 (1968).
122. Norton, L.A. Incorporation of thymidine-methyl-H3 and glycine-2-H3 in the nail matrix and bed of humans. *J. Invest. Dermatol.* **56**, 61–68 (1971).
123. de Berker, D. and Angus, B. Proliferative compartments in the normal nail unit. *Br. J. Dermatol.* **135**, 555–559 (1996).
124. Samman, P. The ventral nail. *Arch. Dermatol.* **84**, 192–195 (1961).
125. Johnson, M. and Schuster, S. Continuous formation of nail along the nail bed. *Br. J. Dermatol.* **128**, 277–280 (1993).
126. Johnson, M., Comaish, J.S. and Shuster, S. Nail is produced by the normal nail bed: a controversy resolved. *Br. J. Dermatol.* **125**, 27–28 (1991).
127. Heikkilä, H., Stubb, S. and Kiistala, U. Nail growth measurement employing nail indentation: an experimental follow-up study of nail growth in situ. *Clin. Exp. Dermatol.* **121**, 96–99 (1996).
128. de Berker, D. Nail growth measurement by indentation. *Clin. Exp. Dermatol.* **22**, 109 (1997).
129. Nishi, G., Shibata, Y., Tago, K. *et al.* Nail regeneration in digits replanted after amputation through the distal phalanx. *J. Hand Surg.* **21A**, 229–233 (1996).
130. Libbin, R.M. and Neufeld, D.A. Regeneration of the nail bed. *Plast. Reconstr. Surg.* **81**, 1001–1002 (1988).
131. Kligman, A. Why do nails grow out instead of up? *Arch. Dermatol.* **84**, 181–183 (1961).
132. Baran, R. Nail growth direction revisited. *J. Am. Acad. Dermatol.* **4**, 78–83 (1981).
133. Tosti, A., Morelli, R., D'Alessandro, R. and Bassi, F. Carpal tunnel syndrome presenting with ischemic skin lesions, acroosteolysis and nail changes. *J. Am. Acad. Dermatol.* **29**, 287–290 (1993).
134. Baran, R. and Juhlin, L. Bone dependent nail formation. *Br. J. Dermatol.* **114**, 371–375 (1986).
135. Todd, G. and Saxe, N. Idiopathic phalangeal osteolysis. *Arch. Dermatol.* **130**, 759–762 (1994).
136. Gilchrist, M. and Dudley Buxton, L.H. The relation of fingernail growth to nutritional status. *J. Anat.* **9**, 575–582 (1938).
137. Hillman, R. Fingernail growth in the human subject. *Hum. Biol.* **27**, 274–283 (1955).
138. Dawber, R. Fingernail growth in normal and psoriatic subjects. *Br. J. Dermatol.* **82**, 454–457 (1970).
139. Babcock, M.J. Methods for measuring fingernail growth rates in nutritional studies. *J. Nutr.* **55**, 323–336 (1955).
140. Sibinga, M.S. Observations on growth of fingernails in health and disease. *Pediatrics* **24**, 225–233 (1959).
141. de Doncker, P. and Piérard, G.E. Acquired nail beading in patients receiving itraconazoleaan indicator of faster nail growth? A study using optical profilometry. *Clin. Exp. Dermatol.* **19**, 404–406 (1994).
142. Hamilton, J.B., Terada, H. and Mestler, G.E. Studies of growth throughout the lifespan in Japanese: growth and size of nails and their relationship to age, sex heredity and other factors. *J. Gerontol.* **10**, 401–415 (1955).
143. Moffitt, D.L. and de Berker, D.A. Yellow nail syndrome: the nail that grows half as fast grows twice as thick. *Clin. Exp. Dermatol.* **25**, 21–23 (2000).
144. Yu, H.J., Kwon, H.M., Oh, D.H. and Kim, J.S. Is slow nail growth a risk factor for onychomycosis? *Clin. Exp. Dermatol.* **29**, 415–418 (2004).
145. Baran, R. The new oral antifungal drugs in the treatment of the Yellow Nail Syndrome. *Br. J. Dermatol.* **147**, 189–191 (2002).
146. Hewitt, D. and Hillman, R.W. Relation between rate of nail growth in pregnant women and estimated previous general growth rate. *Am. J. Clin. Nutr.* **19**, 436–439 (1966).
147. Bean, W.B. Nail growth. Thirty five years of observation. *Arch. Intern. Med.* **140**, 73–76 (1980).
148. Geoghegan, B., Roberts, D.F. and Sampford, M.R. A possible climatic effect on nail growth. *J. Appl. Physiol.* **13**, 135–138 (1958).
149. Donovan, K.M. Antarctic environment and nail growth. *Br. J. Dermatol.* **96**, 507–510 (1977).
150. Gormly, P.J. and Ledingham, J.E. Nail growth in antarctic conditions. *Aust. J. Dermatol.* **24**, 86–89 (1983).
151. Weismann, K.J.H.S. Beau and his descriptions of transverse depressions on nails. *Br. J. Dermatol.* **97**, 571–572 (1977).
152. Ito, T., Ito, N., Saathoff, M. *et al.* Immunology of the human nail apparatus: the nail matrix is a site of relative immune privilege. *J. Invest. Dermatol.* **125**, 1139–1148 (2005).
153. Dorschner, R.A., Lopez-Garcia, B., Massie, J., Kim, C. and Gallo, R.L. Innate immune defence of the nail unit by antimicrobial peptides. *J. Am. Acad. Dermatol.* **50**, 343–348 (2004).
154. Egawa, M., Ozaki, Y. and Takahashi, M. In vivo measurement of water content of the fingernail and its seasonal change. *Skin Res. Technol.* **12**, 126–132 (2006).
155. Gniadecka, M., Nielsen, O. and Christensen, D. *et al.* Structure of water, proteins and lipids in intact human skin, hair and nail. *J. Invest. Dermatol.* **110**, 393–398 (1998).
156. Gillespie, J.M. and Frenkel, M.J. The diversity of keratins. *Comp. Biochem. Physiol.* **47B**, 339–346 (1974).
157. Marshall, R.C. Genetic variation in the proteins of the human nail. *J. Invest. Dermatol.* **75**, 264–269 (1980).

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

158. Forslind, B. Biophysical studies of the normal nail. *Acta Derm. Venereol.* **50**, 161–168 (1970).
159. Lynch, M.H., O'Guin, W.M., Hardy, C. *et al.* Acidic and basic hair/nail 'hard' keratins: their co-localisation in upper cortical and cuticle cells of the human hair follicle and their relationship to 'soft' keratins. *J. Cell Biol.* **103**, 2593–2606 (1986).
160. Hanukoglu, I. and Fuchs, E. The cDNA sequence of a human epidermal keratin: divergence of sequence but conservation of structure among intermediate filaments. *Cell* **31**, 243–251 (1983).
161. Westgate, G.E., Tidman, N., de Berker, D., Blount, M.A., Philpott, M.P. and Leigh, I.M. Characterisation of LH Tric 1, a new monospecific monoclonal antibody to the hair keratin Ha 1. *Br. J. Dermatol.* **137**, 24–31 (1997).
162. Kitahara, T. and Ogawa, H. The extraction and characterisation of human nail keratin. *J. Dermatol. Sci.* **2**, 402–406 (1991).
163. Nakamura, A., Arimoto, M., Takeuchi, K. and Fujii, T. A rapid extraction procedure of human hair proteins and identification of phosphorylated species. *Biol. Pharm. Bull.* **25**, 569–572 (2002).
164. Brosche, T., Dressler, S. and Platt, D. Age-associated changes in integral cholesterol and cholesterol sulphate concentrations in human scalp hair and finger nail clippings. *Aging (Milano)* **13**, 131–138 (2001).
165. Helmdach, M., Thielitz, A., Ropke, E.M. and Gollnick, H. Age and sex variation in lipid composition of human fingernail plates. *Skin Pharmacol. Appl. Skin Physiol.* **13**, 111–119 (2000).
166. Zaias, N. (ed.) The Nail in Health and Disease, 2nd edn, pp. 12–13. Appleton-Lange, Norwalk, CT (1989).
167. Djaldetti, M., Fishman, P., Harpaz, D. and Lurie, B. X-ray microanalysis of the fingernails in cirrhotic patients. *Dermatologica* **174**, 114–116 (1987).
168. Sirota, L., Straussberg, R., Fishman, P., Dulitzky, F. and Djaldetti, M. X-ray microanalysis of the fingernails in term and preterm infants. *Pediatr. Dermatol.* **5**, 184–186 (1988).
169. Alexiou, D., Koutsclinis, A., Manolidis, C. *et al.* The content of trace elements in fingernails of children. *Dermatology* **160**, 380–382 (1980).
170. Jacobs, A. and Jenkins, D.J. The iron content of finger nails. *Br. J. Dermatol.* **72**, 145–148 (1960).
171. Oimomi, M., Maeda, Y., Hata, F. *et al.* Glycosylation levels of nail proteins in diabetic patients with retinopathy and neuropathy. *Kobe J. Med. Sci.* **31**, 183–188 (1985).
172. Sueki, H., Nozaki, S., Fujisawa, R. *et al.* Glycosylated proteins of skin, nail and hair: application as an index for long-term control of diabetes mellitus. *J. Dermatol.* **16**, 103–110 (1989).
173. Pillay, I., Lyons, D., German, M.J. *et al.* The use of fingernails as a means of assessing bone health: a pilot study. *J. Womens Health (Larchmt)* **14**, 339–344 (2005).
174. Behne, D., Gessner, H. and Kyriakopoulos, A. Information on the selenium status of several body compartments of rats from the selenium concentrations in blood fractions, hair and nails. *J. Trace Elements Med. Biol.* **10**, 174–179 (1996).
175. Rogers, M., Thomas, D.B., Davis, S. *et al.* A case control study of oral cancer and pre-diagnostic concentrations of selenium and zinc in nail tissue. *Int. J. Cancer* **48**, 182–188 (1991).
176. Yoshizawa, K., Willett, W.C., Morris, S.J. *et al.* Study of the prediagnostic selenium levels in toenails and the risk of advanced prostate cancer. *J. Natl Cancer Inst.* **90**, 1219–1224 (1998).
177. Garland, M., Morris, J.S., Colditz, G.A. *et al.* Toenail trace element levels and breast cancer: a prospective study. *Am. J. Epidemiol.* **144**, 653–660 (1996).
178. van den Brandt, P.A., Goldbohm, R.A., vant Veer, P. *et al.* Toenail selenium levels and the risk of breast cancer. *Am. J. Epidemiol.* **140**, 20–26 (1994).
179. Vannucchi, H.F., Varo, R.M., Cunha, D.F. and Marchini, J.S. Assessment of zinc nutritional status of pellagra patients. *Alcohol Alcohol.* **30**, 297–302 (1995).
180. Kaneshige, T., Takagi, K., Nakamura, S. *et al.* Genetic analysis using fingernail DNA. *Nucleic Acid Res.* **20**, 5489–5490 (1992).
181. Tahir, M. and Watson, N. Typing of DNA HLA-DQa alleles extracted from human nail material using polymerase chain reaction. *J. Forensic Sci. CA* **40**, 634–636 (1995).
182. Anderson, T.D., Ross, J.P., Roby, R.K., Lee, D.A. and Holland, M.M. A validation study for the extraction and analysis of DNA from human nail material and its application to forensic casework. *J. Forensic Sci.* **44**, 1053–1056 (1999).
183. Nishiyori, A., Fukuda, K., Sata, M. and Tanikawa, K. HBV DNA can be detected from nail clippings of HBs Ag positive patients. *Kurume Med. J.* **47**, 95–96 (2000).
184. Wilhelm, M., Hafner, D., Lombeck, I. and Ohnesorge, F.K. Monitoring of cadmium, copper, lead and zinc status in young children using toenails: comparison with scalp hair. *Sci. Total Environ.* **103**, 199–207 (1991).
185. Das, D., Chatterjee, A., Badal, K. *et al.* Arsenic in ground water in six districts of West Bengal, India: the biggest arsenic calamity in the world. *Analyst* **120**, 917–924 (1995).
186. Harrington, J., Middaugh, J., Morse, D. and Housworth, J. A survey of a population exposed to high concentrations of arsenic in well water in Fairbanks, Alaska. *Am. J. Epidemiol.* **108**, 377–385 (1978).
187. Karagas, M.R., Morris, J.S. and Weiss, J.E. Toenail samples as an indicator of drinking water arsenic

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

exposure. *Cancer Epidemiol. Biomarkers Prev.* **5**, 849–852 (1996).
188. Whitford, G.M., Sampaio, F.C., Arneberg, P. and von der Fehr, F.R. Fingernail fluoride: a method for monitoring fluoride exposure. *Caries Res.* **33**, 462–467 (1999).
189. Gamelgaard, B. and Anderson, J.R. Determination of nickel in human nails by adsorption differential-pulse voltametry. *Analyst* **110**, 1197–1199 (1985).
190. Suzuki, O., Hattori, H. and Asano, M. Nails as useful materials for detection of metamphetamine or amphetamine abuse. *Forensic Sci. Int.* **24**, 9–16 (1984).
191. Suzuki, S., Inoue, T., Hori, H. and Inayama, S. Analysis of methamphetamine in hair, nail, sweat and saliva by mass fragmentography. *J. Anal. Toxicol.* **13**, 176–178 (1989).
192. Miller, M., Martz, R. and Donnelly, B. Drugs in keratin samples from hair, fingernails and toenails. In: *Second International Meeting on Clinical and Forensic Aspect of Hair Analysis (Abstract)*, Genoa, Italy, June 6–8, p. 39 (1994).
193. Cirimele, V., Kintz, P. and Mangin, P. Detection of amphet-amines in fingernails: an alternative to hair analysis. *Arch Toxicol.* **70**, 68–69 (1995).
194. De Doncker, P. Pharmacokinetics of orally administered antifungals in onychomycosis. *Int. J. Dermatol.* **38**(Suppl. 2), 20–27 (1999).
195. Baden, H.P. The physical properties of nail. *J. Invest. Dermatol.* **55**, 115 (1970).
196. Maloney, M.J. and Paquette, E.G. The physical properties of fingernails. I. Apparatus for physical measurements. *J. Soc. Comp. Chem.* **28**, 415 (1977).
197. Finlay, A.F., Frost, P., Keith, A.C. and Snipes, W. An assessment of factors influencing flexibility of human fingernails. *Br. J. Dermatol.* **103**, 357–365 (1980).
198. Wessel, S., Gniadecka, M., Jemec, G.B. and Wulf, H.C. Hydration of human nails investigated by NIR-FT-Raman spectroscopy. *Biochim. Biophys. Acta* **17**, 210–216 (1999).
199. Zaun, H. Brittle nails. Objective assessment and therapy follow-up. *Hautarzt* **48**, 455–461 (1997).
200. Spruit, D. Effect of nail polish on hydration of the fingernail. *Am. Cosmet. Perfum.* **87**, 57–58 (1972).
201. Farren, L., Shayler, S. and Ennos, A.R. The fracture properties and mechanical design of human fingernails. *J. Exp. Biol.* **207**, 735–741 (2004).
202. Zaun, H. and Becker, H. Die quelleigenschaften von nagelmaterial in natronlauge bei bestimmug mit einer standardisierten methode. *Arztl. Kosmetol.* **6**, 115–119 (1976).
203. Jemec, G.B.E., Agner, T. and Serup, J. Transonychial water loss: relation to sex, age and nail plate thickness. *Br. J. Dermatol.* **121**, 443–446 (1989).
204. Walters, K.A., Flynn, G.L. and Marvel, J.R. Physicochemical characterisation of the human nail: permeation pattern for water and the homologous alcohols and differences with respect to the stratum corneum. *J. Pharm. Pharmacol.* **35**, 28–33 (1983).
205. Kobayashi, Y., Miyamoto, M., Sugibayashi, K. and Morimoto, Y. Enhancing effect of N-acetyl-l-cysteine or 2-mercaptoethanol on the in vitro permeation of 5-fluorouracil or tolnaftate through the human nail plate. *Chem. Pharm. Bull. (Tokyo)* **46**, 1797–1802 (1998).
206. Mertin, D. and Lippold, B.C. In vitro permeability of the human nail and of a keratin membrane from bovine hooves: penetration of chloramphenicol from lipophilic vehicles and a nail lacquer. *J. Pharm. Pharmacol.* **49**, 241–245 (1997).
207. Yang, D., Chaumont, J.P., Makki, S. and Millet-Clerc, J. Un nouveau modèle de simulation pour étudier la pénétration topique díantifongiques en kératine dure in vitro. *J. Mycol. Med.* **7**, 195–198 (1997).
208. Pittrof, F., Gerhards, J. and Erni, W. Loceryl nail lacquera realization of a new galenical approach to onychomycosis therapy. *Clin. Exp. Dermatol.* **17**(Suppl. 1), 26–28 (1992).
209. Walters, K.A., Flynn, G.L. and Marvel, J.R. Pressure sealed apparatus for measuring nail plate permeability. *J. Invest. Dermatol.* **1**. 76–79 (1981).
210. Kronauer, C., Gfesser, M., Ring, J. and Abeck, D. Transonychial water loss in healthy and diseased nails. *Acta Derm. Venereol.* **81**, 175–177 (2001).
211. Gunt, H. and Kasting, G.B. Hydration effect on human nail permeability. *J. Cosmet. Sci.* **57**, 183–184 (2006).
212. Mertin, D. and Lippold, B.C. In vitro permeability of the human nail and of a keratin membrane from bovine hooves: influence of the partition coefficient octanol/water and the water solubility of drugs on their permeability and maximum flux. *J. Pharm. Pharmacol.* **49**, 30–34 (1997).
213. Walters, K.A., Flynn, G.L. and Marvel, J.R. Physicochemical characterisation of the human nail: solvent effects on the permeation of homologous alcohols. *J. Pharm. Pharmacol.* **37**, 771–775 (1985).
214. Mertin, D. and Lippold, B.C. In vitro permeability of the human nail and of a keratin membrane from bovine hooves: prediction of the penetration rate of antimycotics through the nail plate and their efficacy. *J. Pharm. Pharmacol.* **49**, 866–872 (1997).
215. Quintanar-Guerrero, D., Ganem-Quintanar, A., Tapia-Olguìn, P. *et al.* The effect of keratolytic agents on the permeability of three imidazole antimycotic drugs through the human nail. *Drug Dev. Ind. Pharm.* **24**, 685–690 (1998).
216. Stüttgen, G. and Bauer, E. Bioavailability, skin and nail penetration of topically applied antimycotics. *Mykosen* **25**, 74–80 (1982).

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

217. Franz, T.J. Absorption of amorolfine through human nail. *Dermatology* **184**(Suppl. 1), 18–20 (1992).
218. Ceschin-Roques, C.G., Hanel, H., Pruja-Bougaret, S.M., Luc, J., Vandermander, J. and Michel, G. Ciclopirox nail lacquer 8%: in vivo penetration into and through nails. *Skin Pharmacol.* **4**, 89–94 (1991).
219. Mensing, H. and Splanemann, V. Evaluation of the antimycotic activity of the pathological substance under the nail after treatment with RO 14–4767 nail lacquer. In: *Proceedings of the 1st EADV Congress*, pp. 21–22. Blackwell Scientific Publications, Oxford (1991).
220. Walters, K.A., Flynn, G.L. and Marvel, J.R. Penetration of the human nail plate: the effects of vehicule pH on the permeation of miconazole. *J. Pharm. Pharmacol.* **37**, 418–419 (1985).
221. Marty, J.P. and Dervault, A.M. Voie percutanée. In: *Therapeutique Dermatologique* (Dubertret, L., ed.), pp. 649–663. Medecine Sciences Flammarion, Paris (1991).
222. Malhotra, G.G. and Zatz, J.L. Investigation of nail permeation enhancement by chemical modification using water as a probe. *J. Pharm. Sci.* **91**, 312–323 (2002).
223. Sidou, F. and Soto, P. A randomized comparison of nail surface remanence of three nail lacquers, containing amorolfine 5%, ciclopirox 8% or tioconazole 28%, in healthy volunteers. *Int. J. Tissue React.* **26**, 17–24 (2004).
224. Kobayashi, Y., Miyamoto, M., Sugibayashi, K. and Morimoto, Y. Drug permeation through the three layers of the human nail plate. *J. Pharm. Pharmacol.* **51**, 271–278 (1999).
225. Mayer, P.R., Couch, R.C., Erickson, M.K., Woodbridge, C.B. and Brazzell, R.K. Topical and systemic absorption of sodium pyrithone following topical application to the nails of the rhesus monkey. *Skin Pharmacol.* **5**, 154–159 (1992).
226. Munro, C.S. and Shuster, S. The route of rapid access of drugs to the distal nail plate. *Acta Derm. Venereol.* **72**, 387–388 (1992).
227. Matthieu, L., de Doncker, P., Cauwenburgh, G. *et al.* Itraconazole penetrates the nail via the nail matrix and the nail bedaan investigation in onychomycosis. *Clin. Exp. Dermatol.* **16**, 374–376 (1991).
228. Onukak, E.E. Squamous cell carcinoma of the nail bed. Diagnosis and therapeutic problems. *Br. J. Surg.* **67**, 893–895 (1980).
229. Baran, R. and Gormley, D.E. Polydactylous Bowen's disease of the nail. *J. Am. Acad. Dermatol.* **17**, 201–204 (1987).
230. Parker, S.G. and Diffey, B.L. The transmission of optical radiation through human nails. *Br. J. Dermatol.* **108**, 11–16 (1983).
231. Yu, R.C.H. and King, C.M. A double blind study of superficial radiotherapy in psoriatic nail dystrophy. *Acta Derm. Venereol.* **72**, 134–136 (1992).
232. Kwang, T.Y., Nee, T.S. and Seng, K.T.H. A therapeutic study of nail psoriasis using electron beams (letter). *Acta Derm. Venereol.* **75**, 90 (1994).
233. Mathis, W.H. and Schulz, M.D. Roentgen diagnosis of glomus tumours. *Radiology* **51**, 71–76 (1948).
234. Camirand, P. and Giroux, J.M. Subungual glomus tumour. Radiological manifestations. *Arch. Dermatol.* **102**, 677–679 (1970).
235. Jablon, M., Horowitz, A. and Bernstein, D.A. Magnetic resonance imaging of a glomus tumour of the finger tip. *J. Hand Surg.* **15A**, 507–509 (1990).
236. Holzberg, M. Glomus tumour of the nail. *Arch. Dermatol.* **128**, 160–162 (1992).
237. Goettmann, S., Drape, J.L., Idy-Peretti, I. *et al.* Magnetic resonance imaging: a new tool in the diagnosis of tumours of the nail apparatus. *Br. J. Dermatol.* **130**, 701–710 (1994).
238. Drapé, J.L. Imaging of the tumors of the perionychium. *Hand Clin.* **18**, 655–670 (2002).
239. Scarpa, R., Soscia, E., Peluso, R. *et al.* Nail and distal interphalangeal joint in psoriatic arthritis. *J. Rheumatol.* **33**, 1315–1319 (2006).
240. Wollina, U., Berger, M. and Karte, K. Calculation of nail plate and nail matrix parameters by 20 MHz ultrasound inhealthy volunteers and patients with skin disease. *Skin Res. Technol.* **7**, 60–64 (2001).
241. Jemec, G.B.E. and Serup, J. Ultrasound of the human nail plate. *Arch. Dermatol.* **125**, 643–646 (1989).
242. Fornage, B.D. Glomus tumours in the fingers: diagnosis with US. *Radiology* **167**, 183–185 (1988).
243. Hirai, T. and Fumiiri, M. Ultrasonic observation of the nail matrix. *Dermatol. Surg.* **21**, 158–161 (1995).
244. Corcuff, P., de Rigal, J., Lévêque, J.L., Makki, S. and Agache, P. Skin relief and ageing. *J. Soc. Cosmet. Chem.* **34**, 177–190 (1983).
245. Grove, G.L., Grove, M.J. and Leyden, J.J. Optical profilometry: an objective method for quantification of facial wrinkles. *J. Am. Acad. Dermatol.* **21**, 631–637 (1989).
246. Nikkels-Tassoudji, N., Piérard-Franchimont, C., de Doncker, P. and Piérard, G.E. Optical profilometry of nail dystrophies. *Dermatology* **190**, 301–304 (1995).
247. Piérard, G.E. and Piérard-Franchimont, C. Dynamics of psoriatic trachyonychia during low dose cyclosporin A treatment: a pilot study on onychochronobiology using optical profilometry. *Dermatology* **192**, 116–119 (1996).
248. Ronger, S., Touzet, S., Ligeron, C. *et al.* Dermoscopic examination of nail pigmentation. *Arch. Dermatol.* **138**, 1327–1333 (2002).

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie

249. Kaufman, S.C., Beuerman, R.W. and Greer, D.L. Confocal microscopy: a new tool for the study of the nail unit. *J. Am. Acad. Dermatol.* **32**, 668–670 (1995).
250. Hongcharu, W., Dwyer, P., Gonzalez, S. and Anderson, R.R. Confirmation of onychomycosis by in vivo confocal microscopy. *J. Am. Acad. Dermatol.* **42**, 214–216 (2000).
251. Goyal, S. and Griffiths, W.A.D. An improved methods of studying fingernail morphometry: application to the early detection of fingernail clubbing. *J. Am. Acad. Dermatol.* **39**, 640–642 (1998).
252. Welzel, J., Lankenau, E., Birngruber, R. and Engelhardt, R. Optical coherence tomography of the human skin. *J. Am. Acad. Dermatol.* **37**, 958–963 (1997).
253. Stavem, P. Instrument for estimation of clubbing. *Lancet* **2**, 7–8 (1959).
254. Bentley, D., Moore, A. and Schwachman, H. Finger clubbing: a quantitative survey by analysis of the shadowgraph. *Lancet* **2**, 164–167 (1976).
255. Regan, G.M., Tagg, B. and Thomson, M.L. Subjective measurement and objective measurement of finger clubbing. *Lancet* **1**, 530–532 (1967).
256. Cudkowicz, P. and Wraith, D.G. An evaluation of the clinical significance of clubbing in common lung disorders. *Br. J. Tuberc. Dis. Chest* **51**, 14–31 (1957).

© 2007 The Authors. Journal compilation
© 2007 Society of Cosmetic Scientists and the Société Française de Cosmétologie