Original Investigation

Comparison of Ex Vivo and In Vivo Dermoscopy in Dermatopathologic Evaluation of Skin Tumors

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IMPORTANCE Ex vivo dermoscopy (EVD) can be a valuable tool in routine diagnostic dermatopathologic evaluation.

OBJECTIVES To compare in vivo dermoscopy (IVD) and EVD and to provide guidance for routine dermatopathologic evaluations.

DESIGN, SETTING, AND PARTICIPANTS This observational study collected 101 consecutive IVD and EVD images of skin tumors from a private dermatology practice from March 1 to September 30, 2013. Four observers (3 dermatologists and 1 dermatopathologist) blinded to the histopathologic diagnoses independently scored and compared the colors, structures, and vessels of EVD images with those of the corresponding IVD images. Data were analyzed from January 1 to March 31, 2014.

MAIN OUTCOMES AND MEASURES Concordance between the EVD and IVD images and gain or loss of colors, structures, and vessels on EVD relative to IVD images.

RESULTS The final analysis included 404 observations of 101 images. The EVD image was generally similar to the corresponding IVD image but clearly darker, with new areas of blue in 130 of 404 observations (32.2%) and white in 100 of 404 observations (24.8%) and loss of red in 283 of 404 observations (70.0%). Most structures were well preserved. New structureless areas were found in 78 of 404 observations of EVD images (19.3%), and new crystalline structures were detected in 68 of 404 observations of EVD images (16.8%). On EVD images, squames and crusts were lost in 56 of 404 observations (13.9%) and 43 of 404 observations (10.6%), respectively. Blood vessels were lost in 142 of 404 observations of EVD images (35.1%).

CONCLUSIONS AND RELEVANCE The EVD image is an important new tool in dermatopathology and may give direction to targeted tissue processing and examination of skin tumors.

JAMA Dermatol. 2016;152(3):312-317. doi:10.1001/jamadermatol.2015.4766
Published online January 6, 2016.

Supplemental content at jamadermatology.com

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In daily practice, most pathology laboratories process skin biopsy specimens without access to the clinical and/or dermoscopic images. In pigmented skin tumors, this information can be crucial to process and diagnose the lesion correctly. Ex vivo dermoscopy (EVD) was first introduced as a valuable tool in dermatopathology by Scope et al,[1] who compared EVD and in vivo dermoscopy (IVD) of 7 lesions and found that EVD reduced errors by aiding selection of areas in which to perform step sectioning. Amin and Fraga[2] reviewed 517 biopsy specimens with EVD and histopathologic correlation. In their study, most features observed in IVD images were also present in EVD images. In 72% of the ambiguous lesions, a more definite diagnosis was assigned after review with the EVD image. In 7.7% of cases, the section margins were reclassified after reviewing the EVD image. Thus, support for the use of EVD for diagnosis and clinicopathologic correlation exists.

With increasingly smaller diameter lesions undergoing biopsy, the focal changes are only visible with dermoscopy; therefore, communication of this dermoscopic information to the pathologist is important. In many dermatopathology laboratories, this communication is often insufficient or totally absent, and one can presume that these suspect areas are often missed with the standard random sectioning technique that examines less than 2% of the tissue.[3] Some surgeons mark areas of interest for the pathologist with a 1-mm punch or use suture marking to direct the pathologist to these suspect areas.[4] However, in our experience, these methods are not satisfactory because they create a disturbance artifact in the tissue specimen. In combination with marking specific and suspected lesions on the biopsy specimen with nail varnish (derm dotting),[5] EVD is a simple and easy method that brings this crucial information to the pathologist and in the slides to be ex-

amined. The practical method and advantages of this new clinicopathologic approach of dermatopathology have been described elsewhere.[5] Using this method, the exact morphologic correlation of dermoscopic characteristics, such as rosettes, can be identified.[6] In a recent study,[7] derm dotting has been used successfully as a marking system on mucosal tissue, namely, in maxillofacial surgery.

Although several studies have compared IVD and EVD,[1,8,9] no systematic comparison by multiple observers in a real-world community setting has been performed, to our knowledge. Therefore, 101 EVD images of consecutive tumoral skin lesions excised in a single dermatology practice were scored independently for different dermoscopic colors, structures, and vessels by 4 independent observers. We compared the results of both techniques to define similarities and relevant differences.

Methods

We included 101 consecutive skin tumors obtained from a private dermatology practice of 2 of us (K.V. and S.L.) from March 1 to September 30, 2013 (**Table 1**). All lesions were documented with in vivo contact polarized dermoscopy (Dermlite 3; 3Gen, Inc) connected to a camera (PowerShot G11; Canon). After fixation in 10% buffered formalin for 12 to 24 hours, we (M.H., I.D.W., and N.D.) obtained contact ex vivo images at the dermatopathology laboratory with a digital camera (Nikon 1 series; Nikon) connected to a polarized dermoscope (Dermlite 3). The ethics committee of University Hospital Ghent approved this study. Because deidentified images and histopathologic diagnoses were examined, the ethics committee waived the need for informed consent.

The IVD and EVD images (JPEG) were scored separately by 3 dermoscopy-experienced dermatologists (K.V., S.L., and L.B.) and 1 dermatopathologist with experience in EVD (M.H.). The 4 observers were blinded to the histopathologic diagnosis (Table 1). The quality of each image was scored as good, moderate, or bad by each observer. After elimination of 1 case (scored as bad quality by all 4 observers), the scores of the remaining 101 IVD and EVD images were compared for significant characteristics, with each observer blinded to the reading of the others (McNemar test). All observations per characteristic (all added scores by item by 4 observers) were subtyped as concordant (no difference between the IVD and EVD images) or discordant. The latter images were subtyped as loss in EVD (ie, the presence of an item on the IVD image but not on the EVD image) and gain in EVD (ie, the presence of an item on the EVD image but not on the IVD image).

Data were analyzed from January 1 to March 31, 2014. We calculated a κ value per item. A significant difference between IVD and EVD was defined as a significant McNemar test result in at least 2 observers and preferentially in 3 or even 4 observers. In a second phase, a similar analysis was performed in the diagnostic subcategories of basal cell carcinomas (BCCs) (n = 31) and invasive melanomas (n = 12). All statistical tests were 2 tailed, and $P < .05$ was considered statistically significant. The analyses were conducted in SPSS software (version 22; IBM).

Table 1. Histopathologic Diagnosis of the 101 Included Skin Tumor Lesions

Type of Tumor	No. of Cases
Melanocytic lesions	47
Congenital nevi	1
Clark or flat nevi	17
Light to moderate dysplasia	16
Severe dysplasia	1
Unna nevi	2
Miescher nevi	1
Acral nevi	1
Blue nevi	5
Spitz or Reed nevi	6
Lentigo maligna	1
Melanoma in situ	1
Invasive melanoma	12
Nonmelanocytic lesions	54
Dermatofibroma	6
Seborrheic keratosis	4
Solar lentigo	3
Angioma	2
Bowen disease	3
Invasive squamous cell carcinoma	3
Basal cell carcinoma	31
Superficial	7
Nodular	21
Infiltrative	3
Pilomatricoma	1
Molluscum contagiosum	1

Results

In total, we included 47 melanocytic and 54 nonmelanocytic lesions (Table 1). For each characteristic, 404 observations were registered. We found concordance in the presence or absence of a feature in 10 549 of 13 736 scored observations (76.8%) and discordance in the remaining 3187 scored observations (23.3%) (**Table 2**).

Colors

In general, colors are well preserved in EVD compared with IVD images. Brown showed concordance between EVD and IVD images in 348 of 404 observations (86.1%). In EVD images, we found a gain of dark brown in 72 of 404 observations (17.8%) (2 observers). A gain of blue on EVD images was seen in 130 of 404 observations (32.2%) and a gain of white in 100 of 404 observations (24.7%) (3 observers for both). In contrast, red disappeared in 283 of 404 observations of EVD images (70.0%) (4 observers).

Similar color differences existed for diagnostic subgroups, especially in BCCs. A gain of brown in BCCs was seen in 22 of 124 observations (17.7%) and a gain of light brown in 24 of 124 observations (19.4%) (2 observers) (eTable 1 in the Supplement). A gain of blue was present in 51 of 124 observations (41.1%) (3 observers) and a gain of white in 41 of 124 observations (33.1%). We found a loss of red in 109 of 124 observations (87.9%) (4 observers). Among invasive melanoma lesions, a gain of white was seen in 17 of 48 observations (35.4%) (2 observers).

Table 2. Results of Scored Characteristics in 101 Tumoral Skin Lesions

Characteristic	No. (%) of Observations (N = 404)			κ Value	No. of Observers With Significant McNemar Test[a]
	Concordant Observations	Loss on EVD	Gain on EVD		
Black	350 (86.6)	23 (5.7)	31 (7.7)	0.65	1
Brown	348 (86.1)	14 (3.5)	42 (10.4)	0.21	2
Dark brown	310 (76.7)	22 (5.4)	72 (17.8)	0.49	2
Light brown	300 (74.3)	59 (14.6)	45 (11.1)	0.10	1
Blue	261 (64.6)	13 (3.2)	130 (32.2)	0.27	4
Red	118 (29.2)	283 (70.0)	3 (0.7)	0.01	4
White	277 (68.6)	27 (6.7)	100 (24.8)	0.35	3
Comedolike openings or milialike cysts	389 (96.3)	7 (1.7)	8 (2.0)	0.59	0
Blue ovoid nests	369 (91.3)	21 (5.2)	14 (3.5)	0.50	0
Red-blue lagoons	395 (97.8)	8 (2.0)	1 (0.2)	0.51	0
Network	352 (87.1)	35 (8.7)	17 (4.2)	0.66	1
Branched streaks	351 (86.9)	34 (8.4)	19 (4.7)	0.64	1
Globules and dots	328 (81.2)	31 (7.7)	45 (11.1)	0.57	1
Structureless areas	304 (75.2)	22 (5.4)	78 (19.3)	0.25	3
Crystalline structures	299 (74.0)	37 (9.2)	68 (16.8)	0.46	2
Yellowish globular structures	403 (99.8)	1 (0.2)	0	0.75	0
Strawberry pattern	390 (96.5)	14 (3.5)	0	0.35	1
White circles	390 (96.5)	9 (2.2)	5 (1.2)	0.24	0
Ulceration	383 (94.8)	16 (4.0)	5 (1.2)	0.35	0
Squames or keratin	318 (78.7)	56 (13.9)	30 (7.4)	0.32	0
Crusts	345 (85.4)	43 (10.6)	16 (4.0)	0.42	2
Pseudonetwork	388 (96.0)	13 (3.2)	3 (0.7)	0.56	0
Hyperpigmented follicular openings	395 (97.8)	8 (2.0)	1 (0.2)	0.71	0
Annular granular pattern	399 (98.8)	5 (1.2)	0	0.58	0
Rhomboidal structures	398 (98.5)	5 (1.2)	1 (0.2)	0.77	0
Peppering	380 (94.1)	7 (1.7)	17 (4.2)	0.63	0
Brainlike or fingerprint appearance	395 (97.8)	7 (1.7)	2 (0.5)	0.36	0
Vessels	252 (62.4)	142 (35.1)	10 (2.5)	0.25	4
Commalike	394 (97.5)	8 (2.0)	2 (0.5)	0.31	0
Dotted	353 (87.4)	43 (10.6)	8 (2.0)	0.02	2
Hairpin	389 (96.3)	10 (2.5)	5 (1.2)	0.36	0
Branched	312 (77.2)	85 (21.0)	7 (1.7)	0.26	4
Linear irregular	337 (83.4)	53 (13.1)	14 (3.5)	0.32	2
Glomerular	397 (98.3)	6 (1.5)	1 (0.2)	0.23	0

Abbreviation: EVD, ex vivo dermoscopy.

[a] All observations per characteristic (all added scores by item by 4 observers) were subtyped as concordant or discordant. A significant difference between in vivo dermoscopy and EVD was defined as a significant McNemar test result in at least 2 observers and preferentially in 3 or even 4 observers.

Loss of red was seen in 35 of 48 observations of melanoma cases (72.9%) (3 observers) (eTable 2 in the Supplement).

Structures

Most structures were well preserved, and the concordance between EVD and IVD images was good, with a 95% CI of 0.50 to 0.77. Structureless areas and crystalline structures were observed more frequently on the EVD images, with a gain in 78 of 404 observations (19.3%) (4 observers) and in 68 of 404 observations (16.8%) (3 observers), respectively. In BCCs, crystalline structures were found on EVD images in 67 of 124 observations (54.0%). Crusts were lost in 13 of 124 observations (10.5%) (3 observers).

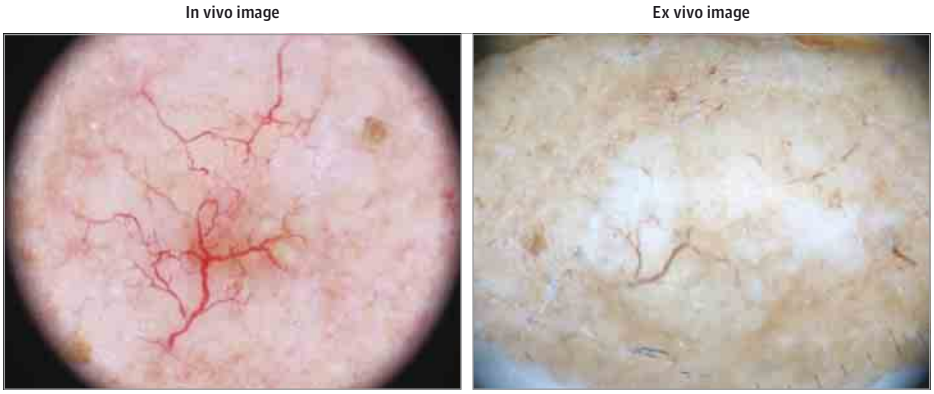
Vascular

Vessels were lost on EVD in 142 of 404 observations (35.1%), which means that in 142 of 212 vessel observations on IVD (67.0%), the vessels disappeared on EVD images (4 observers). In 70 of 212 IVD vessel observations (33.0%), some vascularization was preserved on EVD as light brown, interrupted, branched or irregular linear structures (4 observers). Hairpin vessels, dotted vessels, and glomeruloid vessels more often were lost completely. In BCCs, in vivo vessels were seen in 115 of 124 observations (92.7%), and the vascularization disappeared ex vivo in 76 of 115 observations (66.1%), but partial preservation was seen in 45 of 115 vessel observations (39.1%) (3 observers). Most preservation occurred among the thicker branching vessels.

Discussion

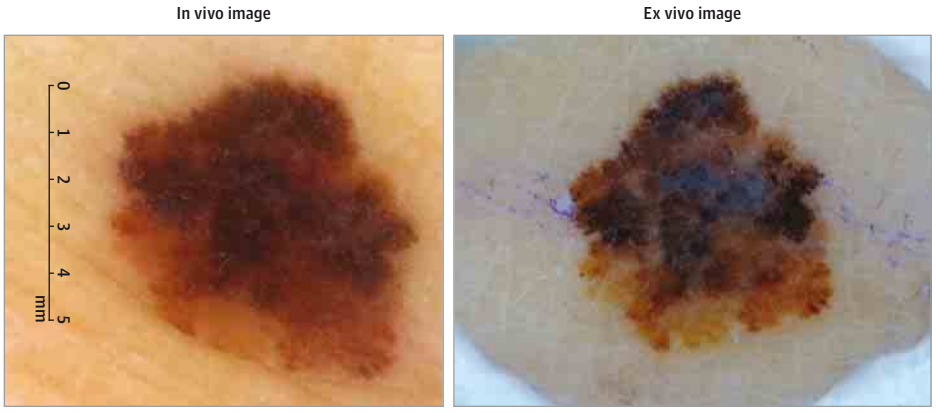
In general, most structures and colors observed on IVD can also be recognized on EVD, confirming earlier findings.[1,2] In our study, we found a general concordance of 76.8% between EVD and IVD images. The most important differences between IVD and EVD images concern the colors. In this comparative study, red disappeared completely on EVD in 70.0% of the cases. In BCCs and melanomas, these percentages were even higher at 87.9% and 72.9%, respectively. Malvehy et al[9] also showed that

Figure 1. Example of Basal Cell Carcinoma (BCC)



The nodular type BCC was located on the back of a man in his 40s. The vessels were partly preserved on ex vivo dermoscopy as light brown curved and branched lines. The ex vivo dermoscopic image showed a new white crystalline structureless area that created a sharper delineation of the tumor compared with the in vivo dermoscopic image.

Figure 2. Example of Melanoma



The lesion (Breslow thickness, 0.5 mm) was located on the abdomen of a woman in her 60s. A new blue area on the ex vivo dermoscopic image disclosed the only invasive area in this melanoma.

colors and structures were comparable between IVD and EVD performed on freshly excised tissue. On the fresh tissue examined with EVD, they found loss of vessels, red, pink, and vascular blush. In the present comparative study, EVD was performed on fixed biopsy specimens.

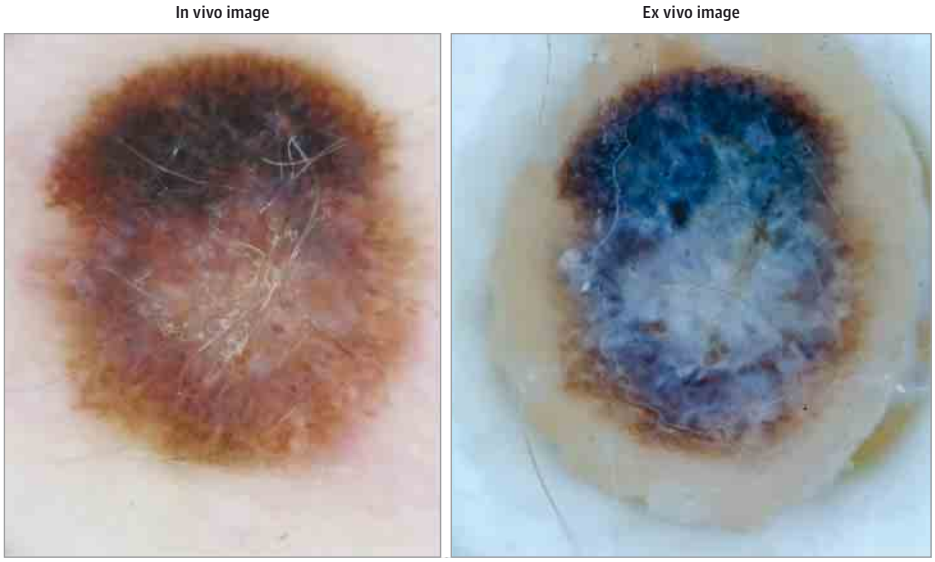
Loss of red was reported earlier by Scope et al.[1] Red disappears mainly by collapse of the vascular structures. However, red also transforms into brown by the fixation process with formaldehyde. In the skin, melanin and hemoglobin are the most important chromophores that selectively absorb different wavelengths. Owing to cross-linking on the molecular level during the formalin fixation process, oxyhemoglobin gradually oxidizes into methemoglobin.[10] The spectrum of light absorption hereby changes, and the red changes into brown.

In accordance with these findings, most vessel structures were no longer discernible on EVD or visible as light brown straight, curved, or clothed lines (**Figure 1**). This change was observed mainly for branched vessels and linear irregular vessels. The visualization of blood vessel remnants as these light brown lines or clods seems to depend on the blood vessel diameter; the larger the vessels, the greater chance that some remnants will be observed on EVD images (eg, dotted, commalike, hairpinlike, and glomerular vessels are more difficult to recognize on EVD images). However, with the shift to brown after fixation, smaller vessel structures are easily overlooked and mistaken for dots, small crusts, or focal erosive areas. This vessel color change may explain in part the observed shift in general from light brown to dark brown with a gain of dark brown in 72 of 404 observations (17.8%) and a loss of light brown in 59 of 404 observations (14.6%).

Another factor that seems to influence the observation of vessel structures with EVD is the fixation time. With shorter fixation times, the chance of observing even smaller vessel remnants on EVD increases. In insufficiently fixed biopsy specimens, some vascular red can still be observed.

In contrast to the red that almost completely disappeared on EVD, the blue and white were observed more frequently, with an increase in 32.2% and 24.8% of the cases, respectively (for BCC, 41.1% and 33.1%, respectively). Blue and white probably become visible or are accentuated by the loss of red during the fixation process. Optical clearing of the tissue by methylene glycol, the hydrated metabolite of formaldehyde, during the fixation process, with reduced scattering of light in collagenous skin tissue; therefore, deeper penetration may also explain the appearance or accentuation of blue and white.[11] Moreover, when this newly discernable blue or white on the EVD image is traced with derm dotting,[5] microscopic examination may reveal important diagnostic areas, such as the foci of invasion in a melanoma lesion that otherwise would be diagnosed as a purely in situ lesion (**Figure 2**).

Figure 3. Example of Spitz Nevus



The lesion was located on the right upper arm of a girl in her teens. A large new blue veil was seen on the ex vivo image.

In addition to an increased number of cases in which blue could be seen on EVD images, the blue present on IVD images was also more prominent on EVD images. This phenomenon was seen through accentuation or appearance of a blue veil (**Figure 3**) and in lesions with a significant presence of deeper dermal melanin pigment (Figure 2). Especially in blue nevi, the diagnostic steel blue color was well preserved, and the image was almost identical to the IVD image.

This gain in white and structureless areas with EVD was accentuated in BCCs, for which 106 of 124 observations (85.5%) showed a white structureless area. In this way, EVD revealed the underlying fibrotic stromal reaction and demonstrated a white structureless area in nearly one-third of the EVD images. Furthermore, the fixation process led to more frequent presence and clearer visualization of crystalline structures in 21 of 124 observations (16.9%). This finding is typical in the case of dermatofibromas and BCCs. The ex vivo image of the dermatofibromas with the stellar white center and crystalline structures hereby becomes very diagnostic. In BCCs, crystalline structures were seen in 54.0% of the EVD images, an increase of 16.1% compared with the IVD images. In some cases of mainly superficial BCCs, these changes created a very different image with a zone of new white, crystalline structures and sharper demarcation (Figure 1). This change could be very useful for proper orientation and evaluation of free margins in BCCs. Other structural elements that can show variation include crusts that tend to disappear on EVD images, especially in BCCs. This tendency is probably owing to detachment of these structures during the fixation and manipulation of the biopsy specimen.

A limitation of this study was the use of 2 different camera systems (Canon vs Nikon) to obtain the IVD and EVD images. In daily practice of IVD, however, different systems are used. Although this finding may have accounted for subtle differences, the observers had the experience that the reported differences between IVD and EVD could not be allocated to the different camera systems. As described in the introduction, our conclusions are in line with the results of other reports. Although the present study pointed out the benefits of EVD for the diagnostic process of dermatopathology, this study did not assess the implications of adding EVD to the potentially improved pathologic diagnostic accuracy. Future research will examine the implications for the diagnostic process.

Conclusions

Ex vivo dermoscopy images are broadly similar to IVD images but also have clear differences and the potential to improve the pathologic diagnosis of skin tumors by providing direction to target tissue for processing and examination. Training in dermoscopy is required for the pathologist and the technical staff. More research is needed to determine the exact effect of this new method on margin evaluation and on the accuracy of diagnosis and appropriate staging of melanoma.

ARTICLE INFORMATION

Accepted for Publication: October 8, 2015.

Published Online: January 6, 2016. doi:10.1001/jamadermatol.2015.4766.

Author Contributions: Drs Haspeslagh and Noë had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Haspeslagh, Vossaert, Lanssens, Brochez.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Haspeslagh, Noë, Degryse, Brochez.
Critical revision of the manuscript for important intellectual content: Haspeslagh, Vossaert, Lanssens, Hoorens, Chevolet, De Wispelaere, Facchetti, Brochez.
Statistical analysis: Haspeslagh, Noë, Hoorens, Chevolet.

Administrative, technical, or material support: Haspeslagh, Vossaert, Lanssens, Noë, De Wispelaere, Degryse.
Study supervision: Haspeslagh, Vossaert, Brochez.

Conflict of Interest Disclosures: None reported.

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NOTABLE NOTES

The Softest Rock on Earth

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The name for talc, a sheer white mineral, is derived from the Greek word *talq*, which means "pure." It is the softest rock on earth. Talc sculptures, vessels, and seals decorated with mythical creatures and animals created by the Mohenjo-Daro and Harappo craftsmen have been found that date back to over 5000 years ago. These craftsmen usually heated their talc, generating a hard, luminous surface.[1] Talc was also used in exquisite Chinese carvings as well as Assyrian cylindrical signets and seals. The ancient Egyptians created scarabs and amulets out of talc, which they commonly varnished with a blue, glossy finish.[2] In addition, talc sculptures from 1100 CE have been discovered in Belur, Halebid and Sravanabelagola, India. In 1890, the powdered form of talc—talcum powder—was found to relieve skin irritations caused by Johnson & Johnson's medicated plasters and was soon used in many other plaster mixes. Johnson & Johnson also realized that talc mitigated diaper rash, leading to the invention of Johnson's Baby Powder in 1894, which is still used today.[1]

We now know that talc, $Mg_3Si_4O_{10}(OH)_2$ or $H_2Mg_3(SiO_3)_4$, consists of hydrated magnesium silicate,[1] although some substitutions of aluminum, iron, or manganese can occur.[3] Talc is formed from soapstone, which is made of talc and other minerals, including mica, quartz, and iron. Over time, the soapstone naturally transforms into steatite and pure talc. Talc is nonporous, chemically inert, lamellar, and does not stain or burn.[1]

Although talc deposits exist throughout the world, the talc mined in each deposit has unique chemical and morphologic characteristics that make it best suited for different domestic and industrial applications.[3] Talc is primarily used in the ceramic and paper industries, although 5% of all consumed talc is used in cosmetics.[1] As a result of weak van der Waals forces, talc's crystalline sheets are able to easily slide past one another, creating a lubricant that moisturizes skin. These sheets are also very flexible yet durable, enabling talc to soften and smoothen skin. Talc's stratified sheets and its translucency allow it to cover up blemishes with just a thin coat. Because talc can be found as a solid, semisolid, or liquid matrix, talc is used in cosmetics that are solid, like antiperspirants and lipsticks; semisolid, such as blushes and eyeshadows; and liquid, like creams and lotions.[3] Talc is also used in a variety of products in other industries, including animal feed, electrical insulation, asphalt roofing, paint, tiles, soap porcelain, pill coating, plastic, rubber, putties, pencils, crayons, counter tops, tailor's chalk, chewing gum, and hard candy.[1,3] Thus, talc is very versatile material with both dermatological and nondermatological applications.

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