

Modern tattoos cause high concentrations of hazardous pigments in skin

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Background: Modern tattoo colourants frequently consist of azo pigments that not only contain multiple impurities but also are originally produced for car paint and the dyeing of consumer goods.

Objective: In order to be able to assess the health risk of tattoos, it is important to determine the pigment concentration in human skin.

Methods: We tattooed excised pigskin and human skin with a common tattoo pigment (Pigment Red 22) under various conditions. After tattooing, we quantitatively extracted the pigment in order to determine the pigment concentration in skin.

Results: The concentration of pigments ranged from about 0.60 to 9.42 mg/cm² of tattooed skin (mean value 2.53 mg/cm²) depending upon the size of the pigment crystals, the pigment concentration applied to the skin surface, and the respective procedure of tattooing.

Conclusion: In conclusion, high concentrations of colourants are injected into the skin during tattooing and based upon this quantification, a risk assessment of tattooing ought to be carried out.

Key words: extraction; pigment concentration; skin; tattoo. © Blackwell Munksgaard, 2008.

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In recent years, the number of tattooed individuals has increased significantly (1, 2), especially among young people (3). In the USA, up to 24% of the population are tattooed (2), whereas in European countries like Germany, approximately 9% of the population and about 12% in the UK have tattoos (4, 5). Nowadays, azo pigments are frequently used for tattooing because of their colour intensity and their longevity. However, azo pigments are primarily manufactured for other purposes such as printing, the painting of cars, and the dyeing of various consumer products. Tattoo colourants are mixtures of pigments (colour) and multiple other ingredients. These colourants usually contain titanium dioxide for lightening the shade (6), precursors and by-products of pigment synthesis, as well as diluents that are used for pigment suspension (7, 8). Tattoo colourants are also applied for permanent make-up on eyelids, eyebrows, and lips (9).

Despite the high incidence of tattoos worldwide, no common legal requirement for listing ingredients has been introduced so far. In Europe, many azo pigments employed in tattoos [e.g. Pigment Red 22 (PR 22)] are not allowed for use in cosmetics because they may be decomposed yielding carcinogenic amines (10).

In the process of tattooing, pigment suspension is deposited in the dermis by piercing the skin with tiny solid needles that are moistened with tattoo colourant. On closer examination, tattooing is a complex procedure that includes various risks for the skin and maybe even for the human body. Pigments and impurities may cause adverse skin reactions at the site of the tattoo (11–22). In addition, part of the colourants is transported to other anatomical locations such as lymph nodes (23, 24). To estimate the risk of any health problems that tattooing might involve for the skin, the pigment concentration in tissue should be

determined – a procedure that has not been attempted so far.

Material and Methods

The azo pigment PR 22 (chemical index 12315, Chemical Abstract Service 6448-95-9) was either synthesized in pure quality (>98%) (25) or purchased as original tattoo pigment (purity approximately 80%, data not shown) (National tattoo supply; Allentown, PA, USA) (7). The pigments were suspended in concentrations of 10% (w/v) and 25% (w/v) in a vehicle of 10% of glycerol (87%; Merck, Darmstadt, Germany) in water (Milli-Q[®] Ultra-pure Water-Purification System; Millipore, Schwalbach, Germany) with the addition of 100 µl of isopropanol (LiChroSolv; Merck) as solubility enhancer.

Pigskin was purchased from a local butchery. Human skin was obtained from skin excisions for other reasons (Department of Surgery, University of Regensburg, Germany). The use of skin was approved by the local Institutional Review Board (IRB). Excision sites were either abdomen or upper arms. The fatty tissue was removed; skin thickness measured approximately 3–4 mm depending on the excision site. Researchers used the tattoo machine, type ‘new lightning’, and typ-

ical tattooing needles (liners and shaders) to inject the colourant into the skin (Deep Colours GmbH, Neuburg am Rhein, Germany). On round needles (liners), tips are arranged in a circle, whereas flat needles (shaders) have linear tips. All needles were solid needles with 4 flat (4F), 4 round (4R), 8 flat (8F), or 8 round (8R) tips. Tattoo artists tend to use round needles with 9 tips (9R). Both needles and the tattoo machine are frequently used for tattooing worldwide.

We applied different methods for skin tattooing. In method A, researchers tattooed pigskin either with synthesized PR 22 or with commercial PR 22 (method B). In method C, professional tattoo artists tattooed pigskin with synthesized PR 22. In method D, researchers tattooed human skin either with commercial PR 22 or with synthesized PR 22 (method E).

A rectangular skin area of about 1 × 3 cm was tattooed (Fig. 1a). We made 3 punch biopsies measuring 5 mm in diameter, extracted the pigment of these samples separately, and calculated the mean value for the resulting 3 values (25). The concentration of skin pigment was calculated as follows: using a 5-mm circular punch biopsy, the skin volume is a cylinder with a skin area of 19.63 mm² times the height of the cylinder. However, only skin material stained with tattoo

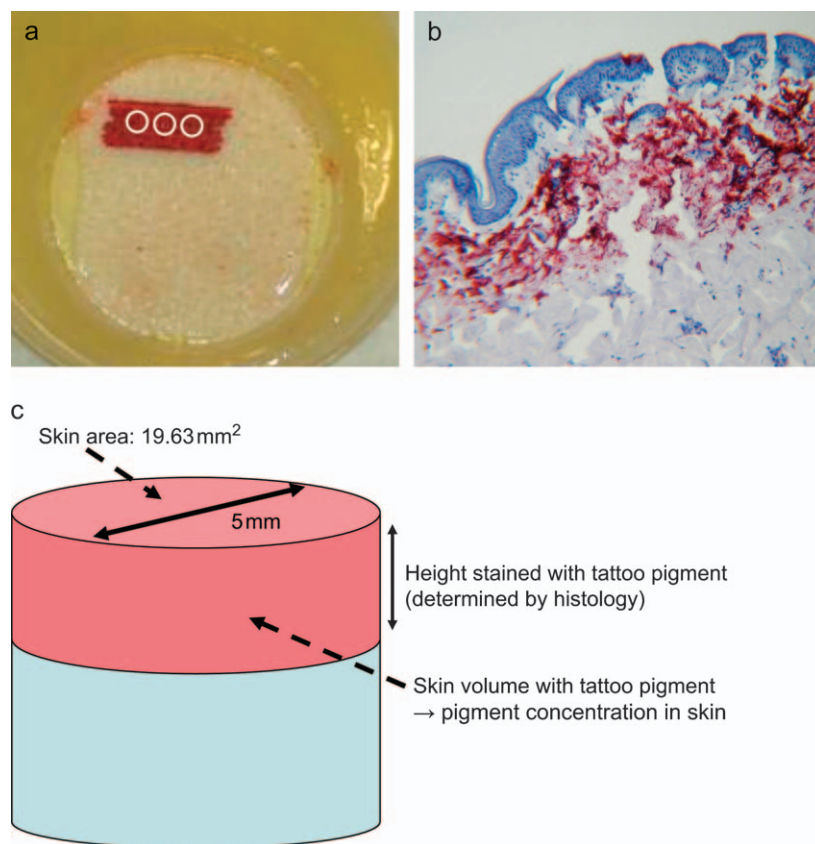


Fig. 1. Skin specimen and histology. The images show recently tattooed pigskin in a stainless steel holder (a), a histological slide of tattooed pigskin (b), and a sketch that illustrates the determination of pigment concentration in skin (c). The white circles in (a) indicate the site of the 3 punch biopsies.

pigment may be used for the analysis. Hence, we histologically determined the cylinder height in order to be able to calculate the pigmented material for each sample. For this height determination, we performed an additional punch biopsy taken from each specimen using standard histological staining (haematoxylin and eosin) and light microscopy. The concentration of skin pigments was calculated as the ratio of the amount of extracted pigment and the volume stained by the pigment (Fig. 1c). For better depiction, we then calculated the pigment concentration as amount of pigment per square centimetre of tattooed skin. For each needle and applied pigment concentration, experiments were performed in triplicate and results were averaged.

Pigment extraction was carried out as previously published (25), which is briefly described as follows. Each punch was powdered in liquid nitrogen, filled into Eppendorf cups (Eppendorf, Wesseling-Berzdorf, Germany) by adding 400 μ l of phosphate-buffered saline (PBS; Biochrom, Berlin, Germany). Proteins were denatured by heating up at 95°C for 20 min according to Gaber et al. (26). After the samples had cooled to room temperature, we added 180 μ l of tissue lysis buffer (buffer ATL) and 15 μ l of proteinase K (Qiagen, Hilden, Germany). Samples were stirred at 55°C for 30 min until complete lysis of the tissue.

The solution resulting from digestion of human skin was added to a test-tube (CELLSTAR[®]; Greiner Bio-one, Frickenhausen, Germany). The volume was increased up to 5 ml with PBS. The aqueous skin phase was stabilized by adding 1 ml of methanol. The resulting mixture was extracted 4 times with 3 ml of methylene chloride.

The total volume was collected in a modified Kuderna-Danish Concentrator (with attached Snyder column). Then, 200 μ l of diethylene glycol dimethyl ether (Diglyme) (Fluka, Deisenhofen, Germany) was added. Finally, the remaining solvent mixture of methylene chloride and methanol was completely evaporated under continuing stirring, elevated temperature (60°C), and a gentle stream of nitrogen (2 bar, 20 min). For the high-performance liquid chromatography (HPLC) analysis, each sample was reconstituted in 1.7 ml of chloroform and 100 μ l solution of internal standard.

The samples were filtered using PTFE filter (CHROMAFIL[®], O-20/15, organic, pore size 0.2 μ m; Machery-Nagel, Düren, Germany). 10 μ l was analysed using a model 1100 HPLC (Agilent Technologies, Waldbronn, Germany) fitted with a C18 analytical column (Phenomenex luna, particle size 3 μ , 150 $\times$ 4.60 mm; Aschaffenburg, Germany). The concentration of PR 22 was deter-

mined by the method of internal standard. For each compound, a calibration factor was determined in a calibration run (single level calibration).

The electron microscopy images were obtained using the transmission technique [transmission electron microscopy (TEM)] (Centre of Electron Microscopy, University of Regensburg, Germany).

Results and Discussion

Based upon a very recently established procedure (25), we quantitatively extracted pigments from tissue after tattooing and, for the first time, determined their respective concentrations in the skin. However, the investigation of tattooing on a scientific level represents a challenge owing to the large variety of tattooing procedures available. Therefore, we used different pigment suspensions and different needles, and both researchers and tattoo artists performed human and pigskin tattooing. This laborious procedure should help to avoid the generation of random values for pigment concentration in skin. Because red tattoo pigments frequently cause allergic skin reactions (11), we used the commonly used red pigment PR22 in our experiments.

Usually, vertically vibrating needles are used for tattooing that inject pigments into the skin with an initial penetration depth of up to 2 mm. Special machines produce this vibration at a frequency of about 30 Hz. Needles exhibit different shapes and number of tips. For tattooing, we initially used original tattoo colourants from the tattoo market (7). However, these colourants usually have a purity of less than 80%. As these impurities may affect the precise recovery experiments, we additionally synthesized PR 22 at a high purity of about 98%.

To determine the pigment concentration in skin, we first used pigskin that is readily available in a standardized form. After performing the same experiments with human skin, we compared extraction results to pigskin experiments. To consider different concentrations of pigments as applied in routine tattoo practice, we used pigment PR 22 at concentrations of 10% (w/v) or 25% (w/v).

Synthesized PR 22 in pigskin

For synthesized PR 22, values for pigment concentration are shown in Table 1 (method A) as amount of pigment per 1 cm² of tattooed skin. Values range from 0.63 to 5.19 mg/cm² depending upon the different concentrations applied to the skin as well as the type and shape of needles used. Histology showed the depth of tattoo

Table 1. Concentrations of pigments in skin^a

Method	Needle size	Applied concentration (w/v) (%)	Amount per tattooed area (mg/cm ²)	RSD (%)
A	8R	10	0.63	13.5
	8R	25	1.42	7.8
	4R	10	1.75	5.9
	4R	25	5.19	15.8
	8F	10	1.02	30.0
	8F	25	2.60	21.6
	4F	10	2.49	4.9
	4F	25	3.44	13.4
B	8R	10	1.90	32.9
	8R	25	3.59	14.1
	4R	10	2.90	45.3
	4R	25	9.42	11.8
C	9R	25	0.60	14.7
D	8R	25	0.95	23.9
E	8R	25	1.69	7.4
Mean value			2.53	17.9

RSD, relative standard deviation.

^aThe amount of PR 22 deposited in 1 cm² pigskin and human skin each. Researchers obtained concentration values in experiments with (A) synthesized and (B) commercial PR 22 in pigskin. The values in (C) stand for experiments performed by tattoo artists using synthesized PR 22 in pigskin. The values for human skin are displayed using commercial (D) or synthesized (E) PR 22. RSD is given for each experimental setting. The last line of the table shows the respective mean of the values for each different setting.

pigments to depend upon skin properties like surface tension. In accordance with our previous report (25), relative standard deviation (RSD) does not depend upon the digestion and extraction scheme but on the properties of the applied pigment suspension.

A needle in a group of 4 tips (4R) results in higher values because the area covered by 1 puncture of 4R is smaller than the area covered by 8R, i.e. more needle injections are necessary for tattooing a certain area of skin when using 4R. Flat needles with 8 tips (8F) result in slightly higher values than 8R. Flat needles show the same correlation between the number of tips and the amount of tattoo pigment injected into the skin.

Comparison of synthesized and commercial PR 22

The injection of commercial PR 22 resulted in higher amounts of pigment in the skin compared with synthesized PR 22 (Table 1, methods A and B), although the commercial colourant contained not only pigment but also impurities up to 20%. Azo pigments tend to agglomerate requiring additional procedures after synthesis. Thus, chemical companies optimize their manufacturing processes that lead to a lower aggregation susceptibility (26).

The pigment synthesized in our laboratory (27) was highly pure PR 22 and did not receive any further treatment. This could explain the different agglomeration and aggregation of primary crystallites. These differences are shown in the TEM pictures (Fig. 2) of commercial or synthesized PR 22 with different mean particle diameters of about 154 and 202 nm, respectively. Hence, the commercial and our synthesized pigments showed a different sedimentation in suspension. We measured a decrease in pigment concentration by 30% in the supernatant of suspension for the synthesized sample, whereas in the commercial sample, the concentration remained unchanged.

This difference suggests a different amount of pigment attached to the needle when dipped into such suspensions. This clearly affects the concentration of pigments injected into the skin but should reflect the various conditions in routine tattooing. The mixture of ingredients in tattoo colourants is neither regulated nor standardized. Despite these facts, the resulting concentrations of pigments in skin are in a confined range regardless the methods used for tattooing (Table 1).

Tattoo artists tattoo pigskin

To compare our scientific procedure with real tattooing, 2 professional tattoo artists were asked to tattoo pigskin with a suspension of pure PR 22 in the concentration of 25% (w/v) using their own equipment including machine and needles.

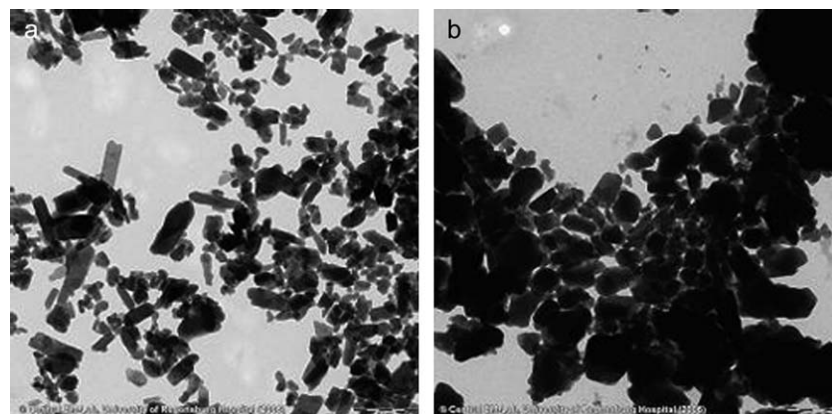


Fig. 2. Size and shape of pigments. Transmission electron microscopy images of commercial (a) and synthesized (b) PR 22 (scale bars = 500 nm).

Table 1 (method C) shows less pigment concentrations in the skin tattooed by tattoo artists but still comparable to the values achieved by researchers.

Human skin

We tattooed various specimens of human skin from different patients. The pigment concentrations determined by extraction matched the range of PR 22 that had been tattooed into pigskin (Table 1, methods D and E). This provides evidence that the nature of tattooed skin has only a minor effect on pigment concentration in skin.

Conclusions

For the first time, the concentration of tattoo pigment in skin has been determined ranging from 0.60 to 9.42 mg/cm². Values and their RSD depend upon the different methods used in our experiments, which should reflect the different conditions in the daily practice of tattooing.

Regardless the different methods used, these values yield an overall mean concentration of 2.53 mg of pigment in 1 cm² of tattooed skin (Table 1). Thus, about 253 mg of azo pigment PR 22 are deposited in the dermis for a typical tattoo covering a skin area of 100 cm². This is an alarming fact because, in Europe, many azo pigments such as PR 22 are forbidden to be used in cosmetics, which are only applied to the skin surface (8). This prohibition is based upon the assumption that such pigments are to some extent absorbed by skin. This absorption may cause health problems, in particular when carcinogenic amines are formed owing to the decomposition of azo pigments. However, in tattooing, hundreds of milligrams are directly injected into the skin.

The medical literature contains multiple case reports (16) on adverse skin reactions after tattooing such as cutaneous pseudolymphoma (15), granulomatous tattoo reactions (12, 17), allergic reactions (19, 21, 22), pseudoepitheliomatous epidermal hyperplasia (13), and even non-melanoma skin cancer (14, 17) or malignant melanoma (18, 20) – albeit skin cancer is assumed to occur only occasionally. The extent to which these adverse reactions are caused by pigments, by other ingredients, or by impurities including bacteria or viruses remains unclear. Unfortunately, physicians are not obliged to report health problems caused by tattoo colourants. As a first step, dermatologists could report on the incidence and the possible reasons of skin problems after tattooing.

In our investigation, we aimed to determine the concentration of a typical tattoo pigment in the

skin immediately after tattooing. This is an important step towards risk assessments with regard to potential health problems caused by tattoo pigments, in particular for the skin. As approximately 20% of colourants represent impurities, these substances should be included in risk assessment. Other risks may result from the possible light-induced decomposition of tattoo pigments in skin. The ultraviolet part of the solar light spectrum may decompose tattoo pigments as shown for Pigment Yellow 74 (28).

The present findings, however, are valid for the pigment used (PR 22). The results should be relevant for other azo pigments showing similar chemical structure, but this has to be confirmed. In light of the large variation of the amount of pigment in the skin (Table 1), the authors would like to emphasize that our results are achieved under *in vitro* conditions. We could not consider the amount of pigment lost through the skin surface during the healing process of skin. For risk assessments, an average exposure from the present experiments should be used.

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