

Colorful Skin – How Tattoos Influence the Antioxidant Balance of the Skin

Gematria Test Lab GmbH

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Introduction

Tattoos are becoming increasingly popular in Germany. According to the market and opinion research institute YouGov in 2021, around a quarter of all people in Germany have tattoos, with the number of tattooed people increasing by around 40 percent over the past 20 years¹⁾. The mechanical, chemical and cosmetic requirements in the tattoo trade are constantly changing in order to improve the longevity of tattoos as well as wound healing and tolerance in the body. For example, the use of products with a high metal and metal oxide content (as a pigment component or impurity) and the intensive use of titanium dioxide (e.g. in the color white) have been known to cause serious and long-lasting skin reactions^{2) 3) 4)}. In the past four years, many tattoo inks and formulation components have therefore been restricted or banned in the European Union due to toxicological reservations in accordance with the REACH regulation⁵⁾. In order to provide REACH-compliant products, organic pigments are increasingly being used. In addition, labels such as “vegan” and “sustainable” are

further drivers of the use of new ingredients. It is often not sufficiently clear what by-products are formed in the skin, e.g. through UV radiation, and what long-term effects may occur. The declaration of ingredients on the products themselves is also repeatedly inadequate and not legally compliant - pigments are listed incorrectly in around every second tattoo ink⁶⁾. Various degradation products are known to occur during the UV-induced decomposition of pigments, such as the carcinogenic 3,3'-dichlorobenzidine and other aromatic amines^{7) 3)}. The frequent addition of dibutyl phthalate (DBP) as a softener is also suspected of causing allergic skin reactions⁸⁾. In addition, the agglomeration of pigments can play a role in the generation of reactive oxygen species⁹⁾. Gematria Test Lab GmbH develops and uses electron spin resonance spectroscopy (ESR spectroscopy) methods for safety and efficacy studies in cosmetics and dermatology. ESR spectroscopy is a physicochemical technique for the characterization of unpaired electrons, e.g. in oxidation and reduction processes. In this article, the properties of tattoo inks of different compositions are investigated and con-

clusions are drawn from them, which should make an important contribution to the production of tattoo inks as well as to the development of corresponding care products. First, a selection of tattoo inks was examined *in-vitro* for radical generation under UV radiation. This was followed by the tattooing of pig ears with these same inks in order to investigate the development of radicals in the skin on the tattooed skin biopsies.

Materials and Methods

Inks and Instruments

Tattoo inks containing pigments from the entire color spectrum were selected for the experiments. These were a yellow, a red, a teal and a black color. Teal contains increased concentrations of preservatives, making it not REACH-compliant. In addition, it contains the colorant CI 74266, for which no information could be found in any database. According to the index it is a phthalocyanine which could be related to the now banned pigments PB 15:3 and PG 7. All other inks are commercial tattoo inks permitted in the EU and contain the pigments listed in Table 1. In addition, all colors contain nickel and chromium (VI) according to the article labeling. An MS-5000 magnettech ESR spectrometer from Freiburg Instruments (now Bruker) was used for all measurements on the skin biopsies, and a magnettech MiniScope MS 300 was used for the *in vitro* analysis of the colors. A Hönle SOL2 UV sun simulator was used to irradiate the skin ($\text{UVA} = 18,81 \text{ mW/cm}^2$; $\text{UVB} = 6,71 \text{ mW/cm}^2$), for the *in-vitro* measurements a Newport UV-Lamp ($\text{UVA} = 12,58 \text{ mW/cm}^2$;

■ **Table 1** Tattoo inks used and the color pigments contained according to the list of ingredients.

Color	Pigment
yellow	CI 21290, CI 77891, CI 12760
red	CI 56110, CI 12475
teal	CI 73000, CI 74266, CI 77891
black	CI 77266

$\text{UVB} = 3,46 \text{ mW/cm}^2$). The pig skin for the tests was obtained from a regional slaughterhouse. Immediately after receipt, the ears were cleaned, shaved and professionally tattooed with the listed colors in a local tattoo studio the following day. The skin was then prepared for the experiments on the premises of Gematria Test Lab GmbH and frozen until further use.

UV-induced free radicals in tattoo inks

The relevant literature reports the generation of free radicals in tattoo inks as a result of decomposition processes caused by UV irradiation, particularly when TiO_2 is used. To test this, five tattoo inks were first mixed 1:10 with deionized water and 0.01 mM PCA (2,2,5,5-tetramethyl pyrrolidine 1-oxyl) was added as a spin probe. The samples were then placed in quartz glass tubes (40 μl), initially without irradiation, and then measured in the ESR spectrometer according to the scheme below after defined irradiation doses. The decrease in the PCA signal intensity was recorded and calculated over the entire irradiation period according to AUC analysis (Area under Curve) to the RP value (Radical Potential). It indicates

how many radicals (in %) were generated in the sample by means of UV radiation ¹⁰⁾.

Tattooed skin biopsies

For the *ex-vivo* examination of the colors and the tattooing process itself, a pig's ear was tattooed with the aforementioned colors. A reference, for which the skin was damaged with a tattoo needle but no color was applied, was also prepared. A cordless Cheyenne SOL Nova with a 5 mm stroke and operating voltage of 9V was used for tattooing, combined with a Kwadron Round Shader 12 needle array with a tip diameter of 0.35 millimeters. Figure 1 shows the ear used with colored tattoo. The skin was then detached from the cartilage, cut into 1x1 cm pieces, sorted by color and treatment, and then deep-frozen.

Skin antioxidant potential

The instrumental antioxidant power of the skin was determined using the test radical 2,2,6,6-tetra-methylpiperidinyloxy (TEMPO). The 1 cm² pieces of skin were placed on filter paper soaked in 40 µL TEMPO and allowed to react

with the test radical for 5 minutes before a 4 mm biopsy was taken and placed in the skin cell for the ESR spectrometer. The degradation of the radical by the skin's own antioxidants was monitored at intervals of 0; 1.5; 3; 5; 7 and 10 minutes. The aim was to investigate the influence of the injury caused by the tattoo needle and the effects of the ink application on the skin's own antioxidant power. Each condition was tested on four biopsies and the mean values and standard deviations were compared with each other.

Radical generation in the skin under UV irradiation

In order to determine the radical generation in the skin by UV irradiation, a 1 cm² piece of skin was placed on a filter paper of the same size containing 27 µL PCA for 10 minutes and a biopsy was then taken. This was placed in the ESR spectrometer and the PCA signal was recorded after defined irradiation intervals in the Hönle sun simulator with UV full spectrum ¹¹⁾. The radical development in tattooed skin due to UV radiation was compared to the radical development in untattooed skin. Here, too, each condition was tested on four pieces of skin.

Results

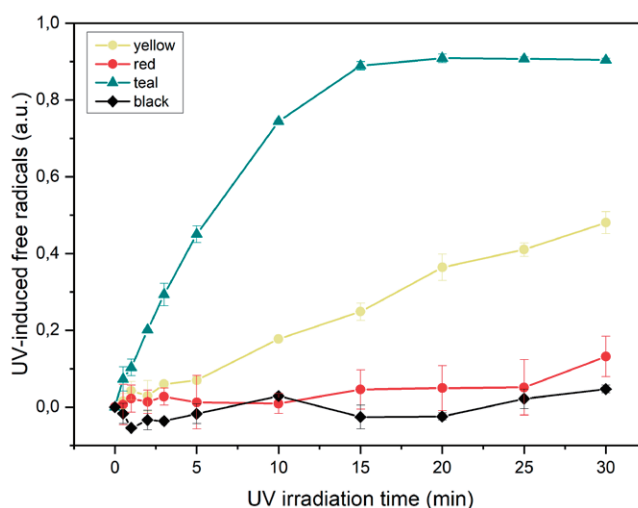
in-vitro radical generation in tattoo inks

The progression of UV-induced radical development is shown in Figure 2. The irradiation of the colors in a quartz glass tube (*in-vitro*, double determination) produced a clear increase in free radicals in the color teal up to saturation, which is reached after about 15 minutes.



■ Fig. 1 Tattooed pig ear.

This is a product with ingredients or ingredient concentrations that are now prohibited under the REACH regulation. At the same time, the color teal also contains TiO_2 (C.I. 77891), which tends to photocatalytic reactions depending on the technological realization and quality. The radical potential could provide information about instabilities in the product, which can lead to severe skin reactions in the body.



■ Fig. 2 Free radical generation in tattoo inks over the course of UV irradiation.

A clear, linear increase in radical generation can also be observed in the color yellow. TiO_2 is also contained here, but with C.I. 12760 and CI 21290 also azo pigments, which are assessed in the literature as potentially unstable and decompose through photodegenerative processes, for example into benzamides or (nitro-) toluenes⁷⁾. The color red is stable up to the section between 25 and 30 minutes, from which an increase in the development of free radicals becomes visible. The color black is inconspicuous over the entire irradiation

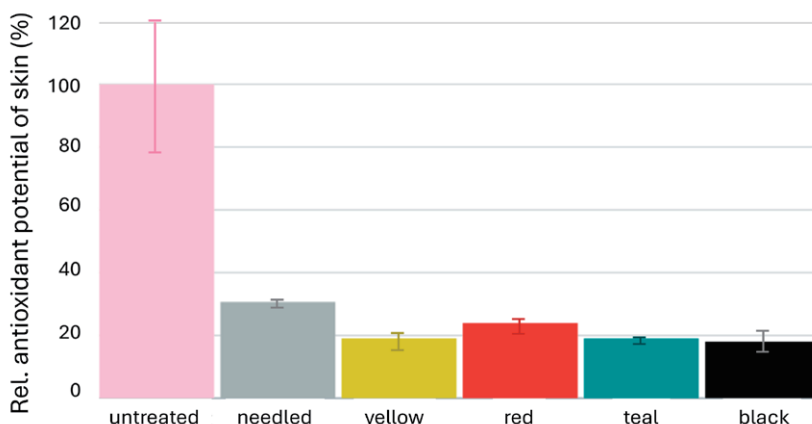
period. According to the AUC calculation, the *in-vitro* free radical development is as shown in Table 2:

Reduction of the skin's intrinsic antioxidant potential through tattooing

When examining the intrinsic antioxidant power of the skin, it becomes apparent how the tattooing process affects the skin. It can be seen that processing the skin with the needle array already leads to a significant reduction in the

■ Table 2 Amount of induced free radicals in tattoo inks.

Color	Induced free radicals in mM	± Standard deviation	RP in %
yellow	$2.54 \cdot 10^{-3}$	$0.03 \cdot 10^{-3}$	25.4
red	$0.42 \cdot 10^{-3}$	$0.51 \cdot 10^{-3}$	4.2
teal	$7.28 \cdot 10^{-3}$	$0.10 \cdot 10^{-3}$	72.8
black	$0.00 \cdot 10^{-3}$	$0.00 \cdot 10^{-3}$	0.0



■ **Fig. 3** Antioxidant power of the skin in relation to untreated skin. The skin injured by the tattoo needle already shows a drop in antioxidant potential of around two thirds. A further drop can be observed when tattoo inks are applied.

antioxidant power to around a third of the original value, as shown in Figure 3.

From the point of view of the injured skin, the introduction of the colors results in a further reduction in antioxidant power. In the case of the color red, this reduction is the lowest at around 20 percent compared to the comparative value of the needled skin. The colors yellow, turquoise and black show very comparable influences with reductions in the antioxidant power of the skin of around 40 percent compared to the needled skin. The results are shown in Figure 4. The differences between the needled skin and the skin tattooed with color are significant.

Influence of UV radiation on skin tattoos

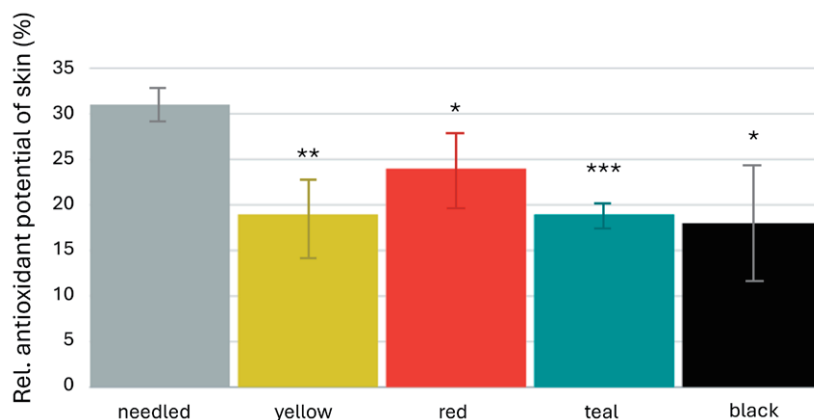
The influence of UV radiation on the skin is shown in Figure 5. The relative number of

UV-induced free radicals is almost three and a half times higher in needled skin than in untreated skin. This also reveals an increased need for sun protection of already injured skin. If the skin treated with tattoo inks is included, the effect is reversed: the pigments injected into the upper layers of the skin create a barrier that reduces the formation of radicals in the skin biopsies.

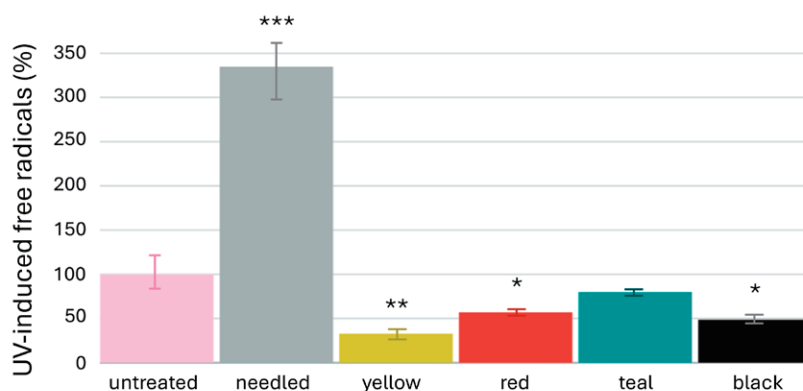
A decomposition of the pigments caused by photodegeneration with the release of free radicals cannot be clearly observed in this experiment.

Discussion

The studies showed that, on the one hand, the photostability of the inks can be measured well *in vitro* and, on the other hand, that the influence of tattooing on the skin is visible using ESR spectroscopy. When measuring the intrinsic



■ Fig. 4 Relative antioxidant power of the skin with significant differences between the skin injured by tattoo needles and the skin treated with tattoo ink (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).



■ Fig. 5 Radical development in the skin as a result of UV irradiation in relation to untreated skin. In all colors except turquoise, a reduced radical development is observed compared to untreated skin, whereas the radical development in needed skin is strongly increased (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

sis antioxidant power of the skin, no connection can initially be established with the results of the *in-vitro* tests. The most conspicuous colors in the development of free radicals under UV irradiation - turquoise and yellow - show

no pronounced influence in the reduction of intrinsic antioxidant power compared to the other colors. Meanwhile, the color red is comparable to black in the *in-vitro* test, but is clearly better tolerated in the *ex-vivo* test. If the re-

sults of the UV irradiation of the skin are now added, a drastic increase in UV-generated free radicals in the injured skin compared to untreated skin can be seen, which can be attributed to the damaged skin barrier. In contrast, the skin tattooed with the colors yellow, red and black shows a significant reduction in the development of free radicals under UV radiation. Only the color turquoise shows no significant differences compared to untreated skin. It can be speculated that a physical skin protection as with the other colors possibly takes place. At the same time, induced photodegradation of the ink, as already seen in the *in-vitro* study, may in turn lead to increased radical development *ex-vivo*, and thus to a non-significant radical reduction compared to untreated skin. Skin reactions usually occur on healed tattoos. During the healing process, the formulation components of the tattoo inks, such as plasticizers and solvents, are released from the wound. A considerable proportion of the injected pigments are also lost during the healing process, as they are introduced into the epidermis instead of the dermis and are therefore shed during the skin's regeneration process within the first two to three weeks after the tattoo. From this point of view, tattooing pig ears to obtain skin biopsies can only be an approximation of the reality of tattoos in human skin. At the same time, the fact that the removal of formulation components by wound or lymphatic fluid has come to a standstill may also make it possible to improve the measurability and thus the risk assessment of these volatile product components.

Summary

Gematria Test Lab GmbH has examined tattoo inks using ESR spectroscopy methods. Using the *in-vitro* method, products with increased UV-induced radical development could be identified, which represents a potential health risk. The *ex-vivo* investigations were carried out on skin biopsies. For this purpose, a pig's ear was damaged with a tattoo needle and selected tattoo colors were applied to other areas. The intrinsic antioxidant power of the skin was already strongly reduced in the damaged skin, and this was further reduced with the application of ink. When the skin is exposed to UV radiation, there is a significant reduction in UV-generated free radicals in skin tattooed with ink compared to untreated skin. Only the color turquoise – a product no longer approved in the EU – shows a similarly high UV-induced development of free radicals as the untreated skin. Injured, non-tattooed skin suffers greatly from UV radiation. The increased need for antioxidants and UV protection for damaged skin can be deduced from the data.

Furthermore, it is unknown to what extent the tattoo product manufacturing industry is aware of the photocatalytic effects of certain pigments, particularly the various technological purity forms or crystal structures of TiO₂. Careful selection of raw materials may be able to significantly reduce the potential health risk at this point.

Acknowledgments

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References:

- 1) Statista Research Department, “Umfrage in Deutschland zu Tattoos nach Altersgruppen 2021”, <https://de.statista.com/statistik/daten/studie/1253983/umfrage/umfrage-in-deutschland-zu-tattoos-nach-altersgruppen/>, 2021, letzter Zugriff: 24.03.2024.
- 2) I. Schreiber, B. Hesse, C. Seim, H. Castillo-Michel, J. Villanova, P. Laux, N. Dreiaek, R. Penning, R. Tucoulou, M. Cotte, and A. Luch, “Synchrotron-based -XRF mapping and -FTIR microscopy enable to look into the fate and effects of tattoo pigments in human skin,” *Scientific Reports*, vol. 7, p. 11395, Sept. 2017.
- 3) M. Agnello and M. Fontana, “Survey on European Studies of the Chemical Characterisation of Tattoo Ink Products and the Measurement of Potentially Harmful Ingredients,” in *Current Problems in Dermatology* (J. Serup, N. Kluger, and W. Bäuml, eds.), vol. 48, pp. 142–151, S. Karger AG, 2015.
- 4) J. Serup, K. Hutton Carlsen, N. Dommershausen, M. Sepehri, B. Hesse, C. Seim, A. Luch, and I. Schreiber, “Identification of pigments related to allergic tattoo reactions in 104 human skin biopsies,” *Contact Dermatitis*, vol. 82, pp. 73–82, Feb. 2020.
- 5) Commission Regulation (EU) 2020/2081 of 14 December 2020 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards substances in tattoo inks or permanent make-up.
- 6) X. Wang, L. Josefsson, S. Meschnark, M. Lind, Emmer, W. Goessler, and Y. S. Hedberg, “Analytical survey of tattoo inks—A chemical and legal perspective with focus on sensitizing substances,” *Contact Dermatitis*, vol. 85, pp. 340–353, Sept. 2021.
- 7) U. Hauri and C. Hohl, “Photostability and Breakdown Products of Pigments Currently Used in Tattoo Inks,” in *Current Problems in Dermatology* (J. Serup, N. Kluger, and W. Bäuml, eds.), vol. 48, pp. 164–169, S. Karger AG, 2015.
- 8) K. Lehner, F. Santarelli, R. Vasold, B. König, M. Landthaler, and W. Bäuml, “Black tattoo inks are a source of problematic substances such as dibutyl phthalate,” *Contact Dermatitis*, vol. 65, pp. 231–238, Oct. 2011.
- 9) T. Hogsberg, N. R. Jacobsen, P. A. Clausen, and J. Serup, “Black tattoo inks induce reactive oxygen species production correlating with aggregation of pigment nanoparticles and product brand but not with the polycyclic aromatic hydrocarbon content,” *Experimental Dermatology*, vol. 22, pp. 464–469, July 2013.
- 10) T. Herrling, M. Seifert, G. Sandig, and K. Jung, “The determination of the radical power – an in vitro test for the evaluation of cosmetic products,” *International Journal of Cosmetic Science*, vol. 38, pp. 232–237, June 2016.
- 11) T. Herrling and K. Jung, “The Radical Status Factor (RSF): a novel metric to characterize skin products,” *International Journal of Cosmetic Science*, vol. 34, pp. 285–290, Aug. 2012.

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Abstract : The regulation of selected pigments in the EU under the REACH regulation has brought innovations to the tattoo ink segment with new organic compounds. At the same time, as the number of tattooed people in Germany increases significantly, so does the range of tattoo care formulations. However, the impact of the injection of pigment-containing formulations on the skin and the resulting need for protection is difficult to quantify. For this reason, Gematria Test Lab GmbH is dedicated to the assessment of UV-induced radical development in tattoo inks and the effects of tattooing on the skin. For this purpose, pig skin was tattooed and the influence on the intrinsic antioxidant power of the skin and the effect of UV radiation on the skin’s intrinsic free radical formation was determined. The aim is to provide test procedures to identify protection and care strategies for skin modified by tattoos and thereby support the development processes of cosmetic care products. At the same time, the methods presented can help to assess the influence of the application of specific tattoo inks and to minimize the damaging effect on the skin through targeted optimization.