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The relation between the depth of pigment disposition and men's skin thickness, the age and tattoo locations on the body

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Summary. The most important function of the skin is to protect the body against harmful mechanical, physical and chemical factors. Its regenerative capacity is sufficient for self-repair in the event of damage, for example, during tattooing, which can be treated as an invasive procedure introducing pigment molecules into skin layers. In the present research on tattoo pigment deposition, the structure of the dermis and epidermis was evaluated using the standard histological technique with hematoxylin and eosin staining. In addition, statistically significant differences between the depth of pigment deposition on the one hand and age, dermis and epidermis thickness and tattoo location on the other were demonstrated.

Key words: Skin, Tattoo, Skin layers

Introduction

From an anatomical point of view, the skin consists of three layers: epidermis, dermis and subcutaneous tissue. The most external is the epidermis, consisting mainly of maturing epidermal cells (keratinocytes), with the following layers: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum. Its thickness is about 0.4-1.5 mm and varies depending on the area of the body. The dermis, whose main components are collagen and elastic fibers, is divided into papillary and reticular layers (Adamski and Kaszuba, 2008). The reticular layer consists of fibers made of collagen types 1 and 3, reticular fibers and elastic fibers, the latter composed of protein with high strength and stretchability. Between reticular layer fibers there are connective tissue cells: fibroblasts (the most abundant), lymphocytes, histiocytes (tissue cells) and mast cells. Collagen and elastic fibers are anchored in a gel-like substance rich in water, i.e. in glycosaminoglycans, one of which is hyaluronic acid. The papillary layer contains small blood vessels, nerve fibers and chaotically arranged collagen and elastic fibers. Finally, subcutaneous tissue is formed by adipose and connective tissue (Wolski and Kędzia, 2019).

Its thickness varies depending on the area of the body, gender or age. The skin of a man is usually thicker than the skin of a woman (Dąbrowska et al., 2018). Any damage to the skin results in a loss of its integrity and physiological balance, which, among others effects, can result in infections. Usually, the skin's natural regenerative capacity is sufficient for self-repair in the event of damage, for example, when a tattoo is made. Therefore, the correct performance of invasive procedures is fundamental for the proper functioning of this organ (Guo and DiPietro, 2010).

Tattooing is an invasive procedure, introducing particles of colored pigment through individual layers of the skin. While performing the tattoo the artist damages the epidermis, breaks through the basement membrane and introduces pigment into the dermis through a needle or group of needles. Initially ink is taken up by keratinocytes, and phagocytic cells (including fibroblasts, macrophages and mast cells) (Barańska et al., 2018; Strandt et al., 2021). Fujita et al. showed that the ink particles and latex beads were endocytosed by fibroblasts and macrophages in the dermis and subcutaneous tissue. Tattoo pigment agglomerates can vary in size (Fujita et al., 1988). Høgsberg et al. citing other publications, quote a particle size range of 10-5000 nm in both in vitro experiments and in vivo human tattoo biopsies (Høgsberg et al., 2011). The injection of ink with a needle damages the natural protective barrier of

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the skin and causes trauma. In a natural way, the body identifies pigment as an intruder and tries to eliminate it, provoking an inflammatory process. Interleukin 10 regulates the acute inflammatory phase of the healing process by suppressing the inflammatory response (Gopee et al., 2005).

The tattoo remains in the skin for many years. Over a long period of time, tattoo ink particles can gradually move into the reticular layer of the dermis. It can give the tattoo a faded and blurred appearance (Grant et al., 2015). Tattoo pigments are deposited in the dermis but some part of them are removed from the skin via the lymphatic system and may be further accumulated in the lymph nodes (Zirkin et al., 2001; Schreiver et al., 2017) or other organs, eg. Kupffer cells in the liver (Sepheri et al., 2017). There is currently little information regarding the disposition of tattoo pigments in the body. They have toxicological potential and pose possible threats due to their chemical reactivity and small size allowing them to pass through cell membranes. Engel et al. described the first quantitative estimate of the amount of tattoo pigments transported from the skin into the body or decomposed by solar or laser radiation. Using mice tattooed with commonly used ink to analyze the transport of pigment particles and tattoo pigment photodecomposition, a recent study reports that 42 days after the tattoo was performed the amount of pigment in the skin decreased by 32±16% (Engel et al., 2010). It is known that tattooed skin contains many solid particles of tattoo pigments. Laser causes the fragmentation of these particles which are then transported away from the skin (Bäumler and Weiß, 2019). The laser energy causes a rapid thermal destruction of the pigment inside dermal cells and it can re-stimulate the skin's immune system. During laser irradiation, toxic metals may additionally release from the pigment. Because the pigment is recognized by the immune system as a foreign material, it can lead to an allergic reaction (Kazandjieva and Tsankov, 2007). Another aspect, commented by Schreiver and Luch (2020), concerns the introduction into the skin of metal remains from tattoo needles containing high amounts of chromium and nickel. Especially, the presence of nickel as a potent contact allergen is an issue of toxicological concern. Both passive and cellular transport through lymph and blood vessels leads to the accumulation of micro and nano metal particles in local lymph nodes. After oxidation, metal ion levels are likely to be sufficient to induce allergies (Schreiver and Luch, 2020).

Tattoos are now so common that they are often treated as a modern decorative element of the body. The more people decide to permanently decorate the body, the greater the number of tattoo complications, which are likely to increase further in the future. In dermopathological practice, tattoos are a common research material due to specific complications. Kazandjieva and Tsankov observed 234 dermatological patients with tattoos, and in 5 (2.1%) cases they have diagnosed adverse reactions to tattoo pigment (Kazandjieva and Tsankov, 2007). In 2017, a case was described of a man who, 5 days after getting a tattoo, bathed in the Gulf of Mexico. It is worth mentioning that he additionally suffered from chronic hepatitis. The tattoo infection was caused by bacteria of the Vibrio vulnificus species, which caused septic shock and death (Hendren et al., 2017). From an epidemiological point of view, tattooing is one of the ways of transmitting many other diseases (including viral and bacterial diseases). Researchers signal that people with tattoos are more likely to be carriers of the hepatitis C virus (Zeuzem et al., 1996; Kazandjieva and Tsankov, 2007; Jafari et al., 2010; Carney et al., 2013).

Unfortunately, it sometimes happens that unsterile diluted ink, repeated use of disposable equipment or lack of sterilization can be the cause of infection. It should also be noted that having a tattoo can further complicate and hinder the clinical diagnosis of melanoma, which can develop within such a tattoo. Its microscopic analysis and evaluation of melanocytic changes can be complicated due to the presence of irregular pigment papules that can mimic melanin. Introduced subcutaneously tattoo pigment is taken in by macrophages, which deliver it to nearby lymph nodes, potentially misleading surgeons and pathologists. Histopathologically, pigment-laden macrophages may look similar to skin areas with melanoma regression (Shinohara et al., 2012). However, Kluger and Koljonen believe that the potential risk of confusing pigment and melanoma should not be a surprise for any surgeon. The question of whether a pathologist may miss metastatic cells due to the concomitant presence of tattoo pigments remains open for discussion (Kluger and Koljonen, 2013).

It is assumed that due to factors such as skin injuries resulting from tattooing, tattoo scars, chronic inflammatory reaction to pigmentation, potential pigment carcinogenesis and exposure to ultraviolet radiation (if the tattoo is exposed to the sun), skin cancer may occur (Patte and Silvis, 2003). However, it is suggested that chronic inflammation that occurs after tattooing is more strongly associated with the onset and development of squamous cell carcinoma (Mataix and Silvestre, 2009). The occurrence of these types of cancer and their relationship to tattooing is still unclear, but they should be included in the list of potential skin complications after tattooing (Junqueira et al., 2017).

The aim of the study was to determine the deposition of tattoo ink in skin cells and the deposition depth of pigment papules depending on the age of a tattooed male person and the dermis and epidermis thickness. The place in the skin where the largest amount of pigment accumulated was also determined.

Materials and methods

The research material was tattoo-covered skin sections collected from 26 men aged 21-60 years sampled from human corpses, from the deltoid area,

chest, abdomen and arm. The consent of the Bioethics Committee at the Medical University of Lublin, Poland, (KE-0254/156/2020) was obtained for the medical experiment. The research concerned the morphology and morphometry of the layers of human tattooed skin and was carried out in cooperation with the Medical University of Lublin (Department of Forensic Medicine with the Laboratory of Forensic Toxicology) and with the University of Rzeszów, Faculty of Medicine (College of Medical Sciences, Department of Histology).

After sampling the material, the tattoo skin sections were preserved using 4% buffered formalin. Then, they were dehydrated in alcohol of increasing concentration, dipped in xylene and immersed in a paraffin block. After making the paraffin block, the skin clippings were cut on a microtome to a thickness of 10μm, then dewaxed by immersion in two xylene baths and hydrated in alcohol with decreasing concentration (absolute alcohol (two times), 96%, 90%, 80% and 70%). In the next stage, the microscopic slide was rinsed with running tap water, and then stained in Harris hematoxylin and eosin - HE (both produced by Sigma-Aldrich, Germany). In the next stage, the clippings were again dehydrated in alcohol with increasing concentration (80%, 90%, 96%, absolute) and dipped in two xylene baths. Prepared in this way, the slide was covered with a coverslip using DPX (trade name for the histology agent for mounting microscope slides; Kolchem, Łódź, Poland). The technique allowed analyzing the visual structure of skin tissues, especially connective and epidermis ones, to determine how deep the pigment from the tattoo penetrates into the dermis.

The assessment of skin layers and the analysis of their individual cellular structures were made using an Olympus optical microscope at 40x and 100x magnification, photographed and measured using the Olympus CellSense Standard software to acquire images from microscopes, but also to archive them and conduct morphometric measurements.

Statistical analysis

Data are expressed as means ± SE. To compare the depth of pigment and the thickness of the dermis and epidermis at the tattoo location, data were analyzed by ANOVA and Tukey's test as a post-hoc test for n=14 chest tattoos, n=8 abdomen tattoos; n=8 deltoid area tattoos, n=12 forearm tattoos (42 tattoos in total, cf. supplementary data set). ; *=p≤0.05 indication of statistical significance compared to abdomen and chest and forearm and deltoid area; *-$=p≤0.05 - indication of significance compared to forearm and chest. For significant dependencies, simple regression and correlation equations were calculated. The significance of correlation and regression coefficients was tested at p≤0.05 on raw data using Statistica 13.

Results

As part of the project, the structure of the dermis and epidermis was assessed with a standard histological technique using HE. In the staining of skin sections, the connective dermis tissue composed of eosin-absorbing collagen fibers is clearly visible in cross section. A well-preserved outline of subcutaneous fat is also clearly distinguished (Figs. 1, 2). The preserved layers of the dermis and their typical structures, such as sebaceous glands, located mainly in the papillary layer, are visible. The layers of the epidermis show an intact structure with a clearly visible layer of keratinized cells. In the

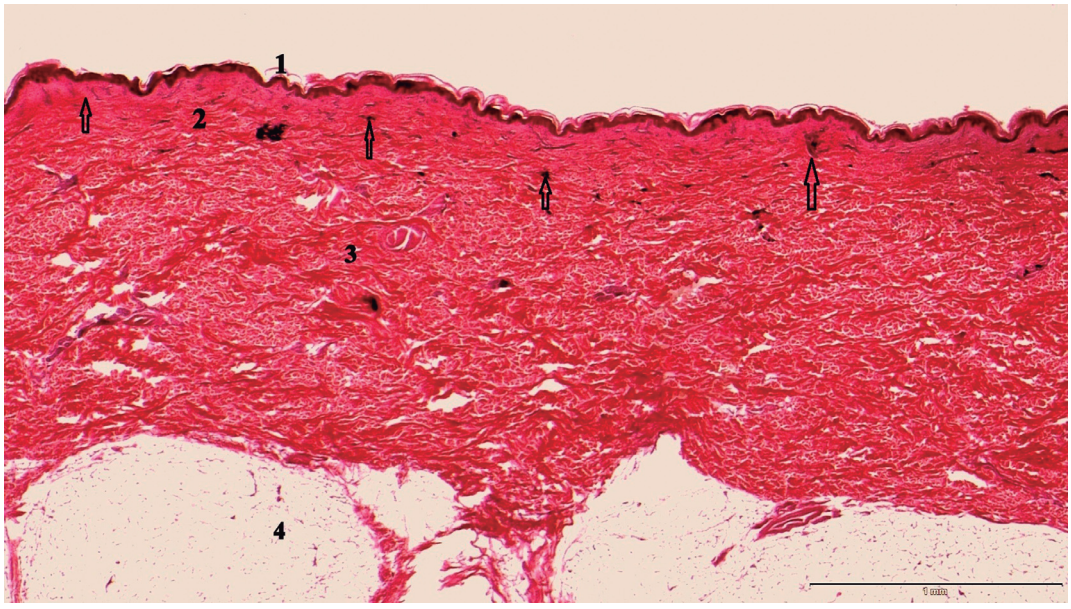


Fig. 1. Tattooed skin section (chest) with preserved subcutaneous tissue with pigment location in the papillary layer: 1, epidermis; 2, papillary layer of the dermis; 3, reticular dermis; 4, subcutaneous tissue; arrow, tattoo pigment.

microscopic images of skin cross sections there are no significant visual differences between samples from different tattoo locations. (Figs. 1-4). In most of the specimens, the tattoo pigment is located under the epidermis, mainly in the papillary layer, and is very rarely visible near the reticular layer (Fig. 4). Noteworthy is the accumulation of pigment near blood vessels (Fig. 3) What should be noted is large differences in the depth of pigment deposition.

The depth of pigment deposition and the dermis and epidermis thickness significantly differ, depending on the tattoo location (Fig. 5A-C). There is also an important association between the depth of the pigment and the dermis and epidermis thickness. As the epidermis and dermis thickness increased, the depth of the deposited pigment also increased. The pigment in the sample from the forearm tattoos deposited the deepest. In the deltoid and chest areas, the pigment deposition was the shallowest. The thickest layer of the epidermis and dermis was observed on the forearm and abdomen, and the thinnest on the deltoid area and chest.

Correlation coefficients indicate that as the dermis thickness increases, the depth of pigment penetrating the skin rises. As the epidermis thickness increases, the

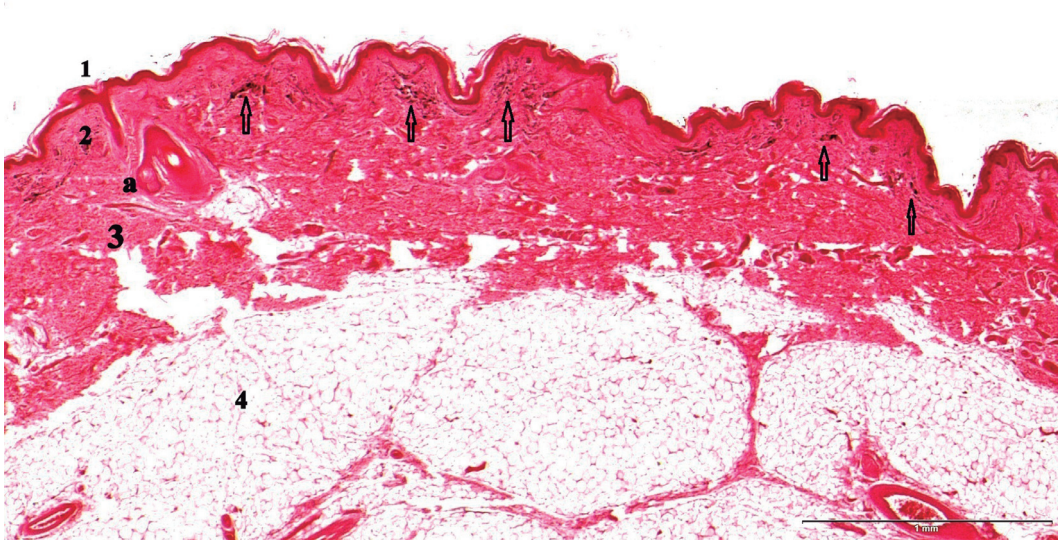


Fig. 2. Tattooed skin section (forearm) with preserved subcutaneous tissue with pigment location in the papillary layer: 1, epidermis; 2, papillary layer of the dermis; 3, reticular layer of the dermis; 4, subcutaneous tissue; a, sebaceous gland; arrow, tattoo pigment.

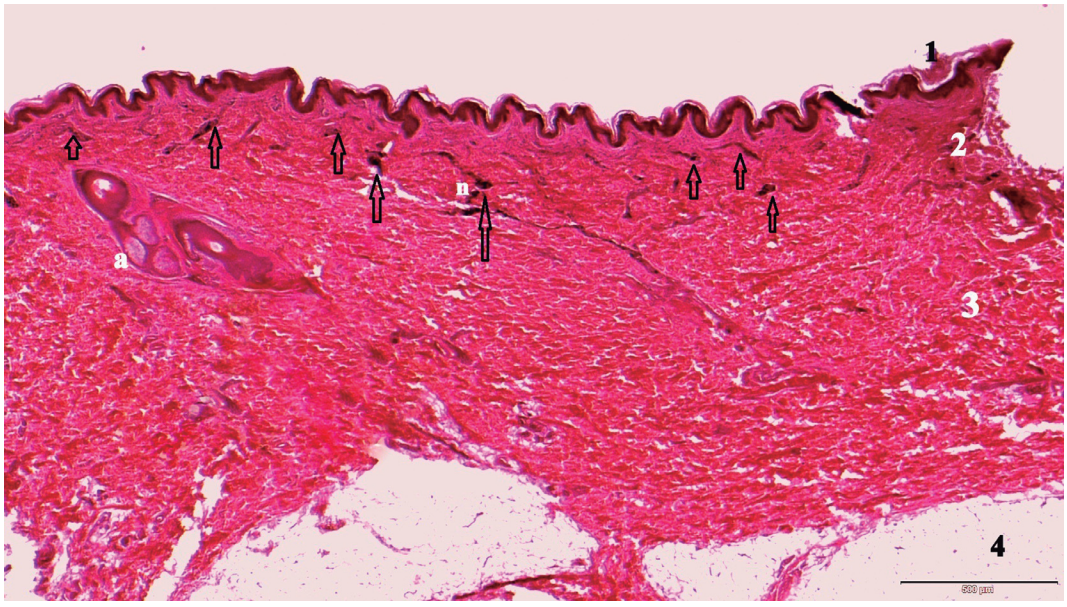


Fig. 3. Tattooed skin section (deltoid area) with accumulation of pigment near blood vessels: 1, epidermis; 2, papillary layer of the dermis; 3, the reticular layer of the dermis; 4, subcutaneous tissue; a, sebaceous gland; n, blood vessel; arrow, pigment from a tattoo.

depth of pigment penetrating the skin rises (Fig. 6).

There is an important association between the depth of pigment deposition on the one hand and the men's age and the dermis and epidermis thickness on the other. With the age of the tattoo, the epidermis and dermis thickness and the depth of pigment deposition decrease significantly (Fig. 7).

Regression equations indicate that as the age of the

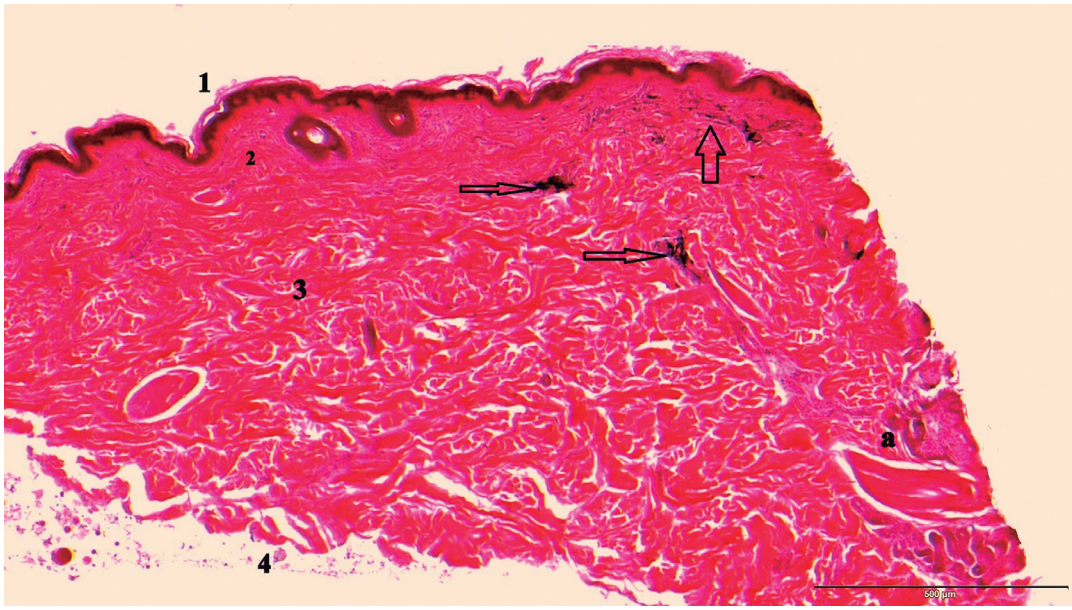
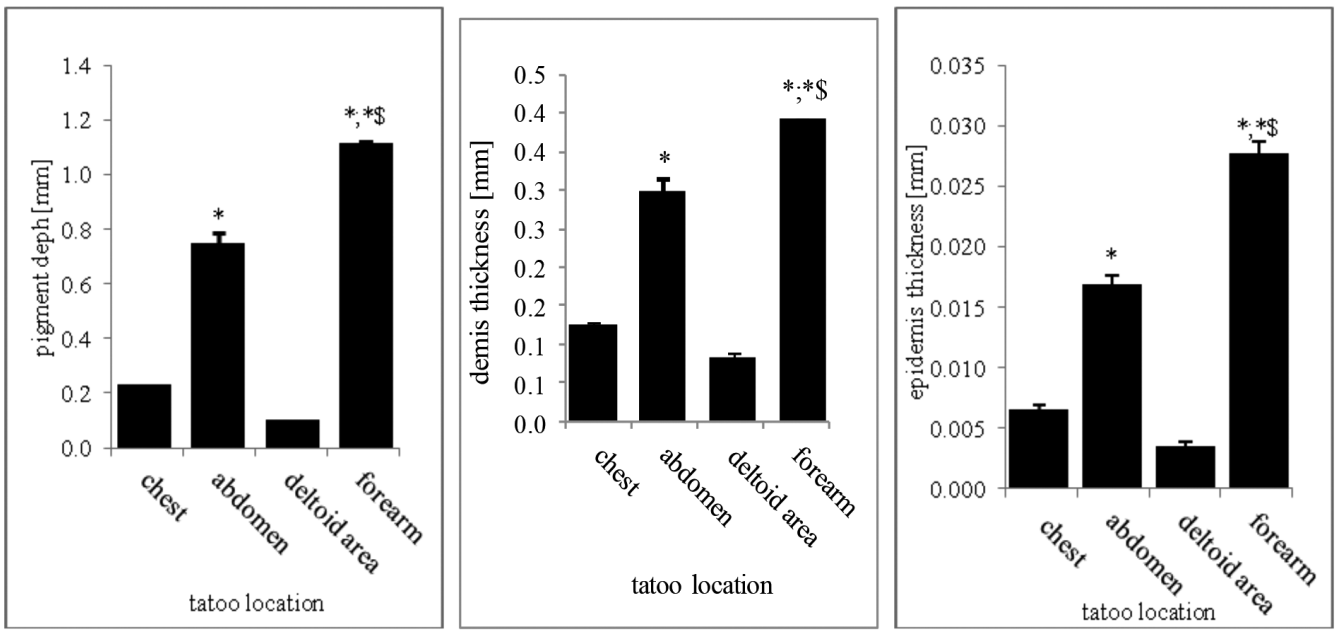


Fig. 4. Tattooed skin section (abdomen) with the tattoo pigment visible near the reticular layer: 1, epidermis; 2, papillary layer of the dermis; 3, the reticular layer of the dermis: 4, subcutaneous tissue; a, sebaceous gland; arrow, pigment from a tattoo.



A B C

Fig. 5. A. The depth of pigment deposition depending on the tattoo location. **B.** The thickness of the dermis depending on the tattoo location. **C.** The thickness of the epidermis depending on on the tattoo location. All values are presented as means ± SE from ANOVA and Tukey's test as a post-hoc test for n=14 chest tattoos, n=8 abdomen tattoos; n=8 deltoid tattoos, n=12 forearm tattoos of 26 men. *: p≤0.05 significance compared to abdomen, chest, forearm and deltoid area; *$: p≤0.05 significance compared to forearm and chest.

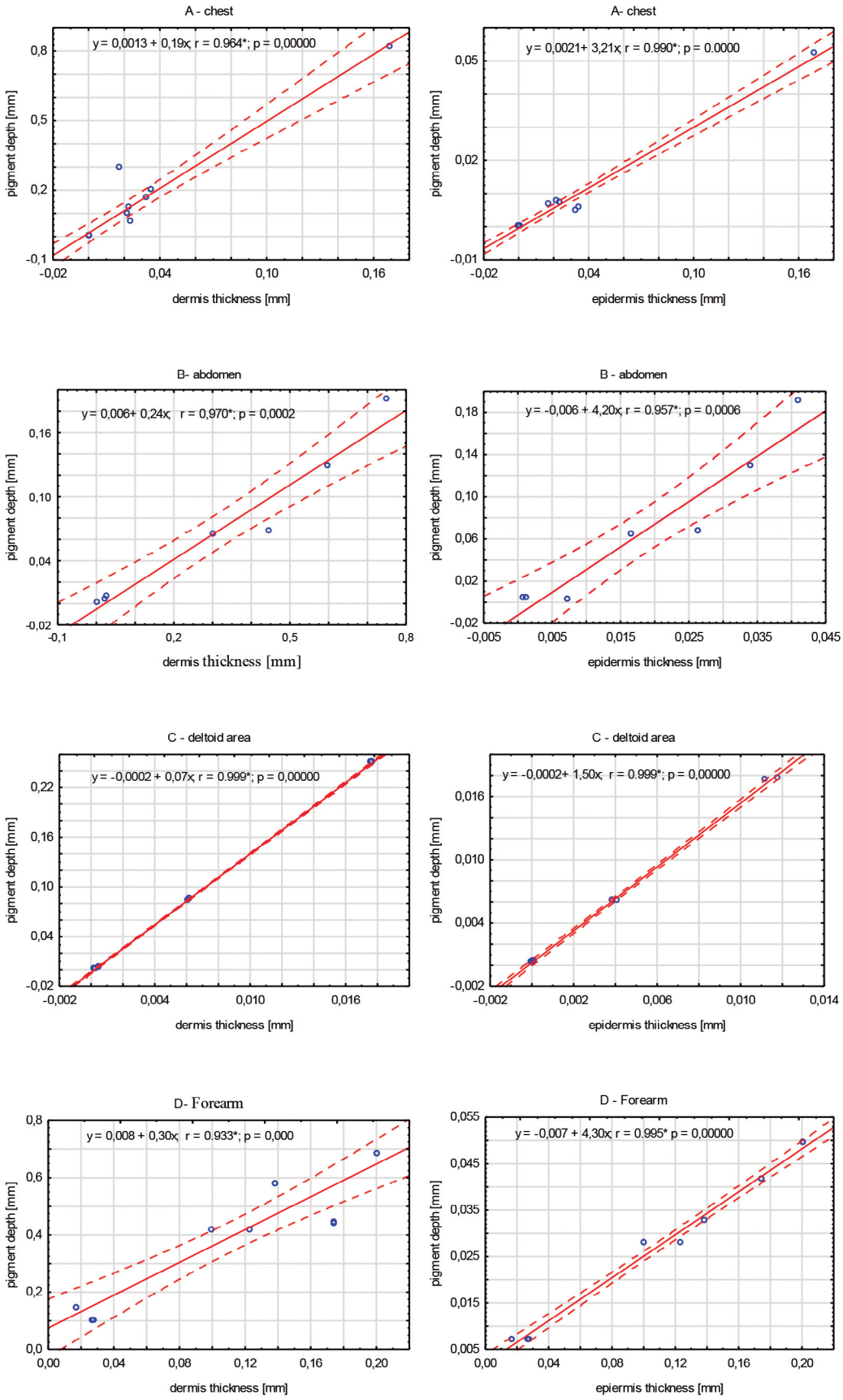


Fig. 6. Correlation graphs between the depth of pigment deposition and the dermis and epidermis thickness. **A.** Chest. **B.** Abdomen. **C.** Deltoid area. **D.** forearm. *: p≤0.05 significance between pigment depth.

tattoo increases by an average of one year, the depth of the pigment decreases by an average of 0.0036 mm, in the dermis by 0.013 mm and in the epidermis by 0.0009 mm.

Discussion

Permanent tattoos are created by injecting solid-containing ink through the epidermis into the dermis, where the pigment is usually deposited in its upper part, up to one third of its thickness. The amount and distribution of pigment depends on such factors as the force and depth of needle insertion, the tattoo machine, the body area and the tattoo age. In our research, we only analyzed the distribution of tattoo pigment in individual layers of the skin. It was found that in most of the analyzed preparations, the tattoo pigment was located under the epidermis, mainly in the papillary layer of the dermis, and was occasionally visible in the reticulate layer. The accumulation of pigment next to the blood vessels is notable. The depth of distribution of the pigment depended on the location of tattoo in the body. Such dependencies were observed in all places where the tattoo was sampled: the deltoid area, chest, abdomen and arm. The pigment deposited the deepest in the sample taken from the tattoo located on the forearm. The depth of pigment deposition was shallowest around the shoulders and chest. According to the authors' observations, tattoos sampled from the deltoid area were well preserved compared to tattoos located on the abdomen or forearm. However, preserving the durability of the tattoo was not the subject of our research. It is worth doing such research in the future. Stability of the tattoo was analyzed by Strand et al. Microscopic analysis showed that a small number of macrophages contained a large amount of pigment, while a large number of dermal fibroblasts contained only a few pigment particles. Our research also revealed the presence of pigment in fibroblasts and macrophages. Investigation of Sleth (2007) and Fujita et al. also shows fibroblasts taking up and storing the ink particles. In the microscopic image of skin cross sections there is a general decrease in pigment aggregates in older tattoos. Changes visible in the histological picture of the epidermis and dermis are part of the aging process. The physiology and pathomorphology of the aging skin indicate mainly sagging and atrophic processes (Engel et al., 2010). Histologically, such skin is characterized not only by atrophy, but also by flattening of interpapilary ridges between dermis and epidermis and a decrease in the number of fibroblasts. Because pigment is enclosed in fibroblasts, by reducing their number, an additional loss of pigment molecules is possible (Strandt et al., 2021). Pigment loss also occurs during the healing process, where part of the pigment molecules are exfoliated, and some, according to the results of the latest research, enter the lymphatic system. The young skin, in which normal immune and regenerative processes take place, eliminates pigment faster than the thin skin of elderly people, where defense processes are disturbed, epidermal structure is flattened and the number of epidermis living layers is smaller. The tattoo remains in the skin for a lifetime, but also undergoes a gradual and slow fading (Engel et al., 2010). An

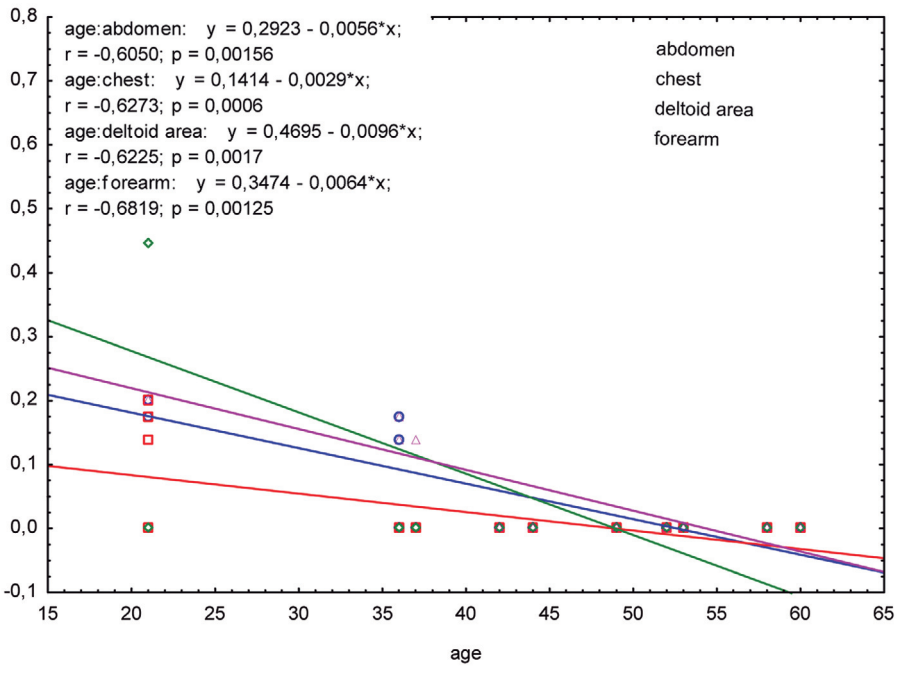


Fig. 7. Regression equations between depth of pigment deposition men's age. *: significant dependence at p≤0.05 between age and pigment depth.

additional factor causing the decomposition and decrease in the number of intradermal pigment papules is ultraviolet radiation. A 2012 study by Shinohara found that the amount and distribution of pigment in the skin also depends on the tattoo age. According to reports, older tattoos show a decrease in the amount of free pigment and its spread into perivascular macrophages. This may be caused by epidermis exfoliation immediately after the ink is injected, transfer of some pigments to the lymphatic system or even intradermal degradation due to ultraviolet radiation, i.e. under the influence of light (Shinohara et al., 2012). A light-microscope analysis of a tattooed patch of skin by Grant and his colleagues (2015), who compared scar tissue and healthy skin tissue, showed greater alignment of collagen fiber in the scar, which, due to the decrease in the biomechanical efficiency of scar tissue, causes ink nanoparticles to settle in, forming clusters at the periphery of the skin collagen network (Grant et al., 2015). In humans, as a result of tattooing, from the first day and for the next 2-4 days, the superficial epidermis rich in pigment exfoliates. Some pigment is gradually assimilated by macrophages, and during the regeneration of the dermis, finally settles in its layers (Sperry, 1992). This is also evidenced by the recent research of Van der Bent et al. (2021), who, determining the depth of inflammation and pigment deposition, found that inflammatory infiltrates and pigment particles were mainly limited to the upper and lower reticular layers of the dermis. In single biopsies, ink particles and inflammatory infiltrates spread throughout the subcutaneous tissue. According to their study, pigment particles can be seen inside macrophages, as well as on their own between collagen bundles in the upper and middle parts of the dermis (Van der Bent et al., 2021).

Conclusions

The present study confirmed the prevailing views on the way pigment particles are deposited in the dermis after tattoo treatment. According to the statistical analysis, there is a significant association between the depth of the deposited pigment and age of tattoo, the thickness of the dermis and epidermis. Such relationships were observed for all regions of sample collection: the deltoid area, chest, abdomen and forearm. Histological observations indicate a large variety of pigment depths. These results may suggest that the removal of these pigments requires an individual approach, for example in the case of laser therapy, where the depth of the pigment particles should be taken into account. Further research on the depth of deposition of the pigment is recommended, with particular significance on its location and possible adverse reactions caused by the tattooing process.

Conflict of interest statement. The authors declare that there are no conflicts of interest.

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