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**Cellular Uptake of Tattoo Ink in Macrophages**

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## **Systemic and Cellular Effects of Tattoo Ink in the Body**

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## Abstract

Tattooing introduces ink particles into the dermis to create permanent art on the skin. Macrophages are phagocytic white blood cells in the dermis. When ink is injected into the dermis during the tattooing process, macrophages take up tattoo ink, and after several weeks to several months, they die and release that pigment for new macrophages to take up. This cycle repeats, which is why tattoos are permanent. However, tattoo ink lacks comprehensive governmental regulation despite its potential health implications. This article explores health risks of tattoo ink, from infections and allergic reactions to potential carcinogenicity, with a special focus on the transformations that tattoo inks can make once they enter the body and, eventually, a macrophage.

**Keywords:** macrophage, tattoo, phagocytosis

## Introduction

One study shows that 32% of Americans have at least one tattoo, with 22% having more than one (Schaeffer & Dinesh, 2023). Despite their prevalence, tattoos pose health risks, including more widely studied outcomes like infections and anaphylaxis, rarer complications like cancer, and less-understood risks like enzymatic transformations or nanoparticle-driven reactions in the body. Although immune cells known as macrophages play a critical role in the longevity of tattoo ink in the dermis, the molecular interactions between tattoo ink and macrophages remains unclear. This article evaluates the potential harmful effects of tattoo ink with a focus on interactions with macrophages.

## Potential Complications from Tattoos

Tattoo inks are often used to create designs on the skin and are even used to create the illusion of permanent makeup when applied to the eyes, lips, or eyebrows. However, infections due to ink contamination, local and systemic allergic reactions to inks, and, rarely, carcinoma, have been reported post-tattoo.

### Lack of FDA Purview

While the Food and Drug Administration (FDA) has not approved any inks for injection, they do not require that tattoo inks undergo an approval process before being brought to market, and there are no requirements for ink manufacturers to disclose the ingredients in their inks to consumers (FDA, 2024). Inks are typically only investigated by the FDA if there is a recall. There were 15 recalls between 2003 and 2021, with 14 being from contamination and one being from allergic reaction (Yoon et al., 2023). This allows tattoo ink manufacturers to provide a vague, incomplete description of ingredients or no ingredient list at all. Ink bottles are not required to display any warnings or to advise a skin patching test to avoid serious allergic reactions. The FDA oversees many other compounds (namely, food and medications) and some may feel it has taken a laissez-faire approach to tattoo ink regulation (Verity et al., 2024).

### Infections from Contamination

According to a 2023 study that screened for contamination of 28 investigated inks, six out of 28 inks contained bacterial contamination from *Bacillus* species as well as *Aerococcus viridans*, *Alloiococcus otitis*, and *Kocuria rosea* (Yoon et al., 2023). A 2021 study investigated 326 patients with adverse

reactions to tattoos and permanent makeup and found that infections occurred in 3.3% of surveyed tattooed patients. Bacterial infections from tattoo ink often stem from *Staphylococcus aureus*, *Clostridium difficile*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*, with rare cases involving *Mycobacterium* species (van der Bent et al., 2021). Fungal infections from *Aspergillus fumigatus* and viral infections from herpes simplex virus and human papilloma virus have also been reported.

### Allergic Reactions

A 2021 study of patients with tattoo ink complications showed that allergic reactions accounted for 52.6% of tattoo ink complications (van der Bent et al., 2021). Inflammation can occur after receiving a tattoo, and this can sometimes become severe and systemic. Redness, swelling, peeling skin, enlarged lymph nodes, granulomas, alopecia, monocular uveitis, excessive eyelid skin, and mounds under the eyes have been reported post-tattoo and permanent makeup administration (Bose et al., 2020; Goldman et al., 2014). Haptenation, or the process in which molecules that are too small to elicit an immune response on their own but are able to when bound to a carrier protein, is one mechanism in which these allergic reactions could occur (Chipinda et al., 2011). Once a hapten binds a protein in the body, it forms a structurally new hapten-protein complex that the immune system recognizes as foreign, which triggers a hypersensitivity reaction against the novel complex. Many haptens are small electrophilic molecules. Some particles within tattoo ink, such as polycyclic aromatic hydrocarbons, can be broken down into electrophilic metabolites by oxidases, and these metabolites could possibly mount a hypersensitivity reaction (Abdel-Shafy et al., 2016).

### **Tattoo Ink: In the Bottle vs. the Body**

Even if tattoo inks are not inherently dangerous compounds when in the bottle, they can be broken down into potentially harmful compounds within the body via enzymes or sunlight. Other pigments begin to release metal ions once they enter cellular environments. Additionally, ink particles that are not necessarily harmful compounds when in microparticle form can begin to pose dangers when they are in nanoparticle form, which is often overlooked by the average tattoo ink consumer.

### Enzymatic Transformation

Cancer occurs when mutations accumulate in successive generations of cells. Since 1938, 36 cases of malignant melanoma in tattooed skin (with 17 from black tattoo ink) have been reported (Leijts et al., 2021). Basal cell carcinoma in tattooed skin has also been reported. There are many potential mechanisms in which tattoo inks may elicit mutations in DNA. Tattoo inks, especially black tattoo inks, have been found to contain potentially carcinogenic compounds, such as polycyclic aromatic hydrocarbons, or PAHs (Regensburger et al., 2010). In the production of carbon black, a pigment that colors many black tattoo inks, PAHs can form and remain in the final ink product (Jacobsen et al., 2015). Enzymes such as oxidases can break down PAHs into even more toxic metabolites such as diol epoxides, dihydrodiols, quinones, and conjugated hydroxyalkyl derivatives that can damage DNA (Abdel-Shafy et al., 2016). Cytochrome P450 (CYP450) enzymes can metabolize PAHs into carcinogenic compounds that can directly interact with DNA (Jacobsen & Clausen, 2015). One study found that upon exposure to light, PAHs can absorb radiation and produce singlet oxygens, or compounds that can oxidize lipids and proteins, damaging cells and tissues (Regensburger et al., 2010). PAHs are lipophilic and can be stored in fat and slowly released, and they can potentially suppress the immune system (Abdel-Shafy et al., 2016). Other tattoo pigments such as red mono-azo pigments can yield potentially carcinogenic aromatic amine

byproducts upon photodegradation from sunlight, including 2-amino-4-nitrotoluene, o-toluidine, and 3,3'-dichlorobenzidine (Hauri et al., 2015). Whether directly (by causing damage to DNA) or indirectly (by oxidizing regulatory proteins or suppressing the immune system), it is quite possible that tattoo ink PAHs could perpetuate cancer in the body.

In contrast, some authors have posited that enzymatic transformation may have a protective effect. Some tattoo inks contain primary aromatic amines (PAAs), which can be detoxified by N-acetyltransferase in cells of the skin, such as keratinocytes (Bonifas et al., 2015; Grohmann et al, 2017; Kochs et al., 2025; Negi et al., 2022). The balance between the protective and deleterious effects of enzymatic transformation warrants more research and may depend on the chemical composition of a particular ink; this includes the relative amount of PAAs, which can be detoxified by Phase II enzymes such as N-acetyltransferase, and PAHs, which can yield carcinogenic metabolites upon transformation by Phase I enzymes such as CYP450. Human skin contains more Phase II enzymes than Phase I enzymes, but different individuals can express varying amounts of N-acetyltransferase in skin, which may further complicate the spectrum of effects than can result from enzymatic transformation of tattoo ink in the body (Bonifas et al., 2015; van Amerongen et al., 2021).

### Metal Release

One 2022 study found that metal ions were released from inks upon macrophagic exposure to zinc and cobalt-based tattoo pigments such as Pigment Blue 28 (PB28, or cobalt aluminate), Pigment Violet 14 (PV14, or cobalt phosphate), Pigment White 4 (PW4, or zinc oxide), and Pigment Green 19 (PG19, or cobalt zinc oxide and deep cobalt green) once the pigments entered the acidic environment of a macrophage (Devicic et al., 2022). TNF- $\alpha$  (an inflammatory cytokine) production by macrophages increased upon exposure to the pigments, and PG19, PB28, and PW4 decreased macrophage phagocytosis.

### Nanoparticles

Even the tattoo ink particles that are not inherently carcinogenic can pose dangers when they enter the body if they are in the nanoparticle form. Nanoparticles are particles smaller than 100 nanometers (Grant et al., 2015). Tattoo inks, especially black tattoo inks, have been found to contain nanoparticles (Høgsberg et al., 2011). Nanoparticles are small enough that they may be able to penetrate cell nuclei and cause damage (American Chemical Society Meeting Newsroom, 2022). Zinc oxide nanoparticles (which can be found in some tattoo inks) cause impaired mitochondrial and cytoskeletal functioning, DNA damage, and reduced phagocytic capacity in macrophages (Aude-Garcia et al., 2016). Copper (II) oxide tattoo ink nanoparticles are toxic to human keratinocytes and fibroblasts (Battistini et al., 2025). It is also worthy to note that carbon black nanoparticles in some, but not all, black tattoo inks, have been found to induce the production of reactive oxygen species (ROS), which are compounds that can cause damage and mutations in cells (Cao et al., 2014).

### **Tattoo Ink Travel in the Body**

The scope of ink particle exposure in the body can be systemic: ink particles and compounds have been found not only in the skin, but also in other locations in the bodies of those who were tattooed. PAHs, for instance, have been found in the skin and regional lymph nodes of deceased people with black tattoos (Lehner et al., 2014). Tattoo pigment particles such as organic pigments, titanium dioxide, and various heavy metals such as aluminum, chromium, iron, nickel, and copper have also been found in the

skin and lymph nodes of tattooed donor bodies (Schreiver et al., 2017). One study that examined tattooed, euthanized mice found the presence of black and red tattoo particles in skin, lymph nodes, and liver Kupffer cells (Sepehri et al., 2017). This demonstrates that ink particles do not simply remain in the dermis, but actually travel within the body to reach other organs. Ink particles may enter into the blood or lymphatic vessels once in the dermis, especially during the needle puncture process of tattooing, and then they may subsequently travel in the bloodstream on their own or within immune cells. Monocytes, circulating white blood cells, have been shown to be effective at taking up ink, which provides one mechanism of ink travel throughout the body (Lin et al., 2023). Ink particles can persist in the body (and eventually reach lymph nodes and/or the liver) if they agglomerate and form particles large enough to escape glomerular filtration into the urine (Sepehri et al., 2017).

### **Ink Entry into Macrophages**

Tattoo artists have exploited the properties of macrophages, long-lived phagocytic white blood cells that reside in body tissues, to create permanent art on the skin (Parham, 2021). Using needles, tattoo artists can puncture the skin and deposit ink into the dermis, where resident macrophages can take-up the pigment (Grant et al., 2015; Baranska et al., 2018). These macrophages, stained with deposited pigment, remain in the dermis and create the appearance of a tattoo design. When these macrophage cells eventually die, they release the pigment they absorbed, and the pigment is soon taken up by new macrophages. This cyclic “pigment capture-release-recapture” model explains why tattoos persist through the duration of the human life span (Baranska et al., 2018). Macrophages have a significantly greater ability to take up ink particles as compared to other cells in the dermis, such as fibroblasts (Strandt et al., 2021).

### **Macrophage Uptake Pathways**

Macrophages are a first-line of defense against infection as they serve to non-specifically engulf and degrade pathogens such as bacteria. Additionally, they scavenge dead cells, secrete pro-inflammatory cytokines to combat infection, and present antigen (Parham, 2021). They have receptors on their cell surface that recognize many exogenous molecules common to bacteria. Once bound to these molecules, the macrophage’s cell membrane envelopes the pathogen and brings it into the cell inside a vesicle known as a phagosome through a process known as phagocytosis (Parham, 2021). The phagosome fuses with a lysosome (a cell organelle filled with degradative enzymes) to form a phagolysosome, where the pathogen is degraded. Most of the degraded pathogen particles, known as antigen, are exocytosed from the macrophage, but some of the molecules remain in the macrophage within vesicles. These vesicles fuse with other vesicles that contain a protein known as major histocompatibility complex (MHC) II (Parham, 2021). MHC II is produced in the endoplasmic reticulum and is trafficked by the Golgi apparatus. The antigen binds the MHC II molecule, which travels to the cell surface in a vesicle. Once MHC II is on the macrophage cell surface, the macrophage can present the antigen to a helper T-cell to activate it. This pathway of antigen presentation is known as the vesicular or endocytic pathway, as it involves the macrophage endocytosing an extracellular antigen such as a bacterium within a vesicle.

Alternatively, macrophages may be infected with viruses that inject antigen directly into the cytoplasm of the macrophage. In this instance, viral particles are degraded by a proteasome, and these degraded particles enter the endoplasmic reticulum, where they bind MHC I molecules (Parham, 2021). The antigen-MHC I complex is trafficked to the cell surface by the Golgi apparatus. A cytotoxic T-cell

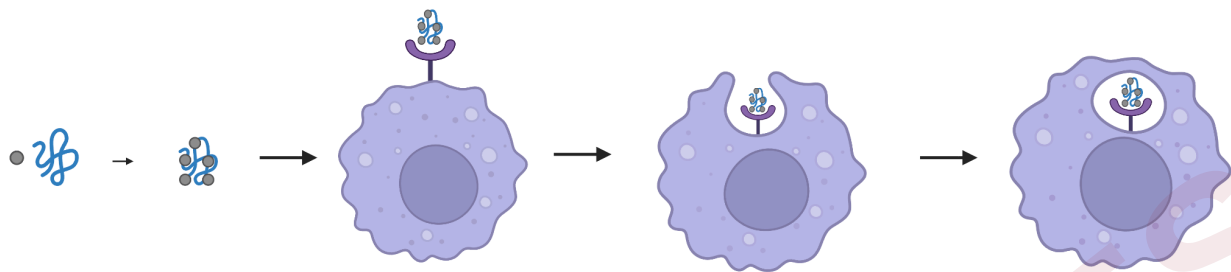
will recognize the antigen and kill the infected macrophage. This pathway is known as the cytosolic pathway of antigen presentation (Parham, 2021).

### Tattoo Ink and Macrophages: What is and is not Known

There have been almost no studies that provide conclusive information about how tattoo ink particles enter macrophages, but some studies include speculations. The authors of one 2016 study on macrophages, while they do not mention tattoo ink, do speculate that zinc oxide nanoparticles are phagocytosed based on the observation of intracellular vesicle formation within the macrophages upon exposure to the nanoparticles (Aude-Garcia et al., 2016). Zinc oxide nanoparticles are found within some tattoo ink pigments such as Pigment White 4 (Devcic et al., 2022). When zinc- and cobalt-based tattoo pigments entered macrophages in a 2022 study, they released metal ions, and researchers used this as evidence to conclude that the pigments likely entered the macrophages through the phagolysosomal pathway, as the ions could have arisen due to pigment exposure to the acidic, oxidizing environments of phagolysosomes (Devcic et al., 2022). Lastly, a 2005 study that investigated macrophagic uptake of ultrafine particles when treated with cytochalasin D found that particles sized 200 nm, but not particles sized 1  $\mu\text{m}$ , were able to enter macrophages when phagocytosis was prevented, but untreated macrophages took up particles of both sizes (Geiser et al., 2005). This finding could provide evidence that nanoparticles (which is the size range for many tattoo pigments) can be taken up by macrophages even when phagocytosis is prevented, which could indicate that some smaller tattoo ink particles could enter the cell through methods other than through phagocytosis. Macrophages do take up significantly more ink particles than non-phagocytic cells such as fibroblasts, so it is quite plausible that phagocytosis is involved in macrophagic ink uptake, but greater investigation into the exact mechanisms of this for different types of particles is necessary (Strandt et al., 2021).

The method of ink particle entry for different tattoo inks is still unknown, but this knowledge could explain whether or not these sometimes harmful compounds enter into the cell with the protection of a vesicle (in the case of phagocytosis or endocytosis) or without the protection of a vesicle (in the case of simple diffusion through the membrane or entry into the cell via channel or transporter proteins). This prompts investigation because, if phagocytosis is a method of uptake, macrophages may alter tattoo ink particles through exposure to an acidic phagolysosome. The effects of this environment on tattoo pigment stability and potential byproduct formation are not yet fully understood. If protected by a vesicle, ink particles may be less likely to have direct access to the nucleus and other organelles, but this merits further research. However, depending on the type of ink particle and how acidic the environment of a certain phagosome is, particle entry into the cell via phagocytosis could be even less protective than via diffusion if harmful byproducts are produced and released from the phagosome. No matter the case, further research on this conundrum is warranted to gain a deeper understanding of the interaction between macrophages and tattoo ink. If ink particles are indeed phagocytosed, the exact mechanisms of this have not been widely published. Haptentation could possibly play a role in ink particle phagocytosis in the body if tattoo ink particles or particle fragments bind to proteins that are recognized by surface macrophage receptors (such as toll-like receptors or scavenger receptors), which would cause them to be engulfed (Figure 1). However, further in vivo experiments are needed to investigate this. Nanoparticles can be opsonized by antibodies and taken up via phagocytosis, as well as through macropinocytosis, endocytosis, and membrane wrapping, so it is possible that this is the case with tattoo ink nanoparticles (Behzadi et al., 2017). As for cell entry without a vesicle, nanoparticles, when hydrophobic, can simply diffuse across lipid bilayers from areas of high concentration to low concentration, and this could be the case for ink

nanoparticles. Hydrophilic nanoparticles can form nano-sized holes in membranes to enter cells, and nanoparticles can also enter cells through channels and transporters (Behzadi et al., 2017).



**Figure 1. Proposed mechanism of haptention of tattoo ink particles.** An ink particle may not be immunogenic when unbound to a protein; However, when bound to a protein, ink particles may be recognized by macrophages. Further experimentation is required to investigate this proposed mechanism.

## Conclusion

Tattooing creates lasting artwork on the skin but also introduces foreign pigments into the skin that can persist for a lifetime and permeate to other areas of the body. Once in the skin, tattoo ink particles are harbored by phagocytic immune cells known as macrophages. Tattoo ink is not inert– it can undergo chemical reactions within the body that maybe make its constituent particles more dangerous. Not only can ink be potentially contaminated with microorganisms or cause allergic reactions, but it also may contain carcinogens and harmful nanoparticles. Understanding the molecular fate of tattoo ink particles once they reach macrophages can better inform researchers of health risks and how to combat them.

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