Tattoo Inks for Optical Biosensing in Interstitial Fluid

Martalu D. Pazos, Yubing Hu,* Yuval Elani, Kathryn L. Browning, Nan Jiang, and Ali K. Yetisen

The persistence of traditional tattoo inks presents an advantage for continuous and long-term health monitoring in point of care devices. The replacement of tattoo pigments with optical biosensors aims a promising alternative for monitoring blood biomarkers. Tattoo inks functionalization enables the control of interstitial biomarkers with correlated concentrations in plasma, to diagnose diseases, evaluate progression, and prevent complications associated with physiological disorders or medication mismatches. The specific biomarkers in interstitial fluid provide a new source of information, especially for skin diseases. The study of tattoo inks displays insufficient regulation in their composition, a lack of reports of the related complications, and a need for further studies on their degradation kinetics. This review focuses on tattoo optical biosensors for monitoring dermal interstitial biomarkers and discusses the clinical advantages and main challenges for in vivo implantation. Tattoo functionalization provides a minimally invasive, reversible, biocompatible, real-time sensing with long-term permanence and multiplexing capabilities for the control, diagnosis, and prevention of illness; it enables self-controlling management by the patient, but also the possibility of sending the records to the doctor.

1. Introduction

Tattoos are a ubiquitous mode of self-expression, with a prevalence in European and American population around 12% and 24%, respectively.[1] Optical biosensors in the last decade have developed extensively in different fields, including therapeutics, environment, and security.[2] Placing a sensor inside the dermis as a tattoo to be in contact with interstitial fluid (ISF) represents a powerful platform for continuous monitoring of biomarkers reducing environmental interferences and the use of optical biosensors avoid the necessity of electrical power nor recharging.

Among the applications of functionalized tattoos are the monitoring of different physiological biomarkers such as, glucose,[3] sodium,[4] albumin,[5] oxygen,[6] cortisol,[7] lactate,[8] pH,[9] nucleic acids,[10] but also external ones such as environmental contaminants[11] or toxic food additives.[12] Functionalized tattoos are possible to release drugs,[13] vaccinate,[14] and also monitor levels of alcohol ingested,[15] body temperature,[16] electrocardiogram measurements,[17] prevention of sun overexposure,[18] and even incorporate identity documents or credit card details into our skin.[19] Functionalized tattoos for ISFs sensing provide effective solutions to monitor and record the evolution of a patient’s disease with the capability of multiplexing, a considerable advantage in metabolic diseases such as diabetes where more than one parameter is normally unbalanced, like pH and glucose. Preventing future complications means an improvement in personal quality of life and economic savings for society.

1.1. Tattoos and Ink Composition

Tattoos can be classified as either i) temporary (e.g., transfer tattoos, henna, or Genipa Americana), ii) semi-permanent (e.g., make-up); and iii) permanent tattoos. The first electric tattoo machine was designed by Thomas Edison in 1877 (Figure 1A) who was trying to design an electric pen,[20] there were no significant advances to modern machines. Tattoo machines can be classified into three types: Coil, rotary, and pneumatic.[21] The tattoo needle is an aggregation of individual needles, and they can be classified into two groups: Lines or shadows. The needles have their nomenclature according to the needle diameter, number of needles grouped, and their configuration (Figure 1A).[20–22] The location, depth, and inclination of the sensor injection are managed with the tattoo needle. The sensor is dispensed on the skin’s surface, the needle creates a hole in the skin and, when the needle is removed, the vacuum generated forces the ink into the hole. Tattoo pigments are injected into the dermis between 0.4

M. D. Pazos, Y. Hu, Y. Elani, A. K. Yetisen
Department of Chemical Engineering
South Kensington Campus
Imperial College London
London SW7 2AZ, UK
E-mail: yubing.hu@imperial.ac.uk

M. D. Pazos, K. L. Browning
Leo Foundation Center for Cutaneous Drug Delivery
Department of Pharmacy
Copenhagen University
Copenhagen 2100, Denmark

N. Jiang
West China School of Basic Medical Sciences & Forensic Medicine
Sichuan University
Chengdu 610041, China

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and 2.2 mm in depth; the ideal penetration depth is 2.0 mm as shallower injection rapidly fades while deeper penetration damages the subcutaneous tissue.\(^9\)

Tattoo inks are a dispersion of insoluble pigments in a liquid composed of solvents and additives (Figure 1B). Dyes are unsuitable for tattoo inks as they are soluble and degradable. Pigments are insoluble and widely used in tattoo ink dispersions, they can be classified as organic, inorganic, and carbon black. All pigments are impure; inorganic pigments tend to contain heavy metals; organic contain primary aromatic amines (PAAs) and carbon black pigments have polycyclic aromatic hydrocarbons (PAHs). Other impurities include formaldehyde, parabens, and isothiazolinones.\(^{24}\) Dispersions are thermodynamically unstable systems leading to separation of the phases and sedimentation of the solid phase. Suitable inks must contain particles that do not sediment too quickly and the solvent, usually water, must not be too volatile as to dry before administration. Examples of other solvents that can be added to water to modify the drying properties, to mask odors, and to increase the ink’s wetting, viscosity, and dispersibility include glycerin, propylene glycol, and simple and polyvalent alcohols. They are use in low concentrations to avoid skin irritation. Additives correct unwanted features; their concentration must not be higher than 5% of the ink.\(^{25}\) The binders keep pigment particles together after drying, making the injection of the ink into the skin easier; an example is the polyvinylpyrrolidone.\(^{25}\) Surfactants are added to decrease surface tension between the particles and the fluid phase, an example is nonylphenol. Inks are usually stored for a long time, causing sedimentation of the pigment. To minimize settling, additives to increase the viscosity and thixotropy are also needed.\(^{26}\) an example of a thickening agent is hydroxyethylcellulose. Fillers like barium sulphate are inorganic substances that add volume to the tattoo ink, creating a re-dispersible porous structure which improves storage properties. It is preferable to form flocculated systems rather than agglomerates for long-term storage of inks; the flocculated particles are porous and easy to redisperse since they are weakly bound.\(^{25}\) As the ink is mostly water, the probability of microorganism contamination is high and it is reduced by using preservatives, like parabens. Ink producers offer the same colorant for more than one field (plastics, prints, clothes), therefore, tattoo inks use pigments that have not been produced to be introduced into the dermis.\(^{27}\) Tattoo pigments do not consistently respect the purity and heavy metal maximums set out in the European Union Resolution ResAP (2008) 1 developed by the Council of Europe, the document that currently guides the security of tattoo inks and permanent make-up (PMU) in Europe.\(^{28}\) This resolution should be translated into national legislation, however, only ten EU countries have adopted it.\(^{24,27,28}\) Most European countries forbid to use of ingredients listed in Annex II and IV of the European Commission (EC) No. 1223/2009 on Cosmetic Products\(^{29}\) because they are carcinogenic, mutagenic, reprotoxic, or sensitizing. Manufactures use the preservatives included in Annex V of EU-Regulation 1223/2009 for cosmetic products, however, there are preservatives allowed in the list that should be avoided in tattoos, like mercury-containing preservatives or formaldehyde-releasing substances.\(^{30}\) Although some countries do not have national legislation, the producers have to demonstrate the safety of their components, controlled

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**Figure 1.** Tattoo machines, needles, and inks. A) Types of tattoo machines and tattoo needles. 1. Tomas Edison’s patent No.196747 for his electric pen from 1877. Reproduced with permission.\(^{[23]}\) Copyright 1877, United States Patent Office. 2. Dual-coil tattoo machine. 3. Rotary machine. 4. Pneumatic machine. A-Tip, B-Needle, C-Tube, D-Grip, E-Energy system: Coil/rotative motor/air piston, F-Armature bar, G-Contact screw. 5. Needle types RL-Round liner, RS-Round shader, F-Flat, and M-Magnum M1 weaved, M2 double-stacked, MR curved. B) Composition of tattoo inks.
Figure 2. Chemical components of tattoo pigments. A) Percentage of Ti, Al, Si, Cu, Cr, Fe, Cl, S, C, O, and Mg of 10 different colors from the tattoo ink suppliers: Huck Spaulding Enterprises Inc., Voorheesville, NY and Eberhard Faber Inc., Lewisburg, Tenn. Quantitative electron X-ray microanalysis by energy-dispersive spectrometry. Reproduced with permission.[31] Copyright 2001, American Medical Association. B) Composition in μg g⁻¹ of the metals Cd, Co, Cr, Hg, Mn, Ni, Pb, Sr, V, and in mg g⁻¹ of the metals Al, Ba, Cu, Fe, Sb of 7 different colors from the tattoo ink suppliers: Starbrite-Colors, Millennium Colorworks Inc., Intenze Prod. and Diabolo by Deep Colors. Sector Field Inductively coupled plasma mass spectrometry. Limits of quantification from 0.07 to 10 ng mL⁻¹, Hg was in traces. Reproduced with permission.[34] Copyright 2009, Elsevier.

by the General Product Safety Directive (Directive 2001/95/EC); to follow the labelling demands and registration of chemicals via CLP (Classification, labelling, and packaging) (EC No 1272/2008); and to respect the requirements of registration for the REACH regulation (Regulation, evaluation, authorization and restriction of chemicals) (EC No 1907/2006).[24] A study of the Joint Research Center (JRC) of the EC in 2016 proved the violation of safety limits set by the ResAP(2008)1 by different tattoo brands in Europe. After JRC results, in 2019 the European Chemical Agency (ECHA) proposed a new annex of restrictions on tattoo inks and PMU compositions, evaluated by the Committees for Risk Assessment (RAC) and Socio-Economic Analysis,[24] it constitutes a common European advance toward a European legislative framework. The absence of coordinated analytical procedures, guidelines for risk assessment, and good manufacturing practice requirements, causes that banned components such as aromatic amines, formaldehyde, or benzothiazolinone can be detected in tattoo inks.[29]

The most common elements in tattoo ink composition are oxygen, titanium, and carbon, contained in 73%, 67%, and 67% of pigments, respectively.[31] However, the quantitative electron X-ray microanalysis by energy-dispersive spectrometry does not reliably identify the presence of elements with an atomic number lower than 11, like oxygen. Inorganic titanium dioxide (TiO₂) pigments have a high refractive index, so they scatter light very effectively; they are used for lightening colors or as white ink (Figure 2A). Organic pigments are sorted into two main classes: 1. Azo compounds which contain the group R–N = N–R⁺ and 2.
polycyclic compounds which are composed of linked aromatic rings (except for triphenylmethane) possessing a chromophore through conjugated pi-bonding systems. Carbon black pigments result from the partial burning of heavy petroleum compounds, they usually contain PAHs impurities. Inorganic pigments often contain heavy metals impurities (Ni, Cr, Cu, and Co) from iron oxides while organic pigments contain less impurities being the mercury salts, cadmium salts, chromium oxide, and cobalt oxides the main sensitizing components. Modern inks reduced their content on these substances but still maintaining other impurities. The primary metals found in tattoo inks were Al, Cu, Fe, and Ti. In a sample of 56 inks, allergenic metals like Cr and Ni surpassed the maximum of 1 ppm in 62.5% and 16.1% of the inks, respectively (Figure 2B) as well as, other toxic elements like Cd, Mn, Pb, Sb, and V. Trace amounts of Hg were also detected. Analyzing or comparing inks’ composition is complicated by the massive variation between brands, including inks with equal base color. Inks also contain carcinogenic impurities, it is necessary to develop methods that mimic the effects of ink persistence in the tissue.

The size of tattoo particles ranges from 5 nm to more than 1 μm; therefore, inks contain nanoparticles (NPs) and micro-particles. NPs are often highly reactive as they possess a high surface to volume ratio and can pass membranes, be transported in the blood, and enter tissue cells causing oxidative stress, inflammation, and apoptosis. Carbon black particles start in the manufacturing process with a size of 15–300 nm, but quickly form irreversible agglomerates of 1–100 μm. The tattoo particles observed with transmission electron microscopy (TEM) (Figure 3) were irregular with different shapes, forming agglomerates, aggregates, and precipitates of organic pigments, as shown in Figure 3A for a precipitate of a red azocompound. The lowest presence of metallic NPs was found in white pigments, mainly composed of TiO₂ forming clusters as shown in Figure 3B, C, E, G. Characterizing the size of tattoo particles is essential to determine the size of optical sensors in tattoo functionalization. The persistence of tattoos in the dermis is determined by the size of the pigment. The sensors should mimic the commercial tattoo pigments, being small enough to avoid surgical procedures but large enough to avoid diffusion from the implantation site. Combining different techniques provides a complete measurement of the ink particles’ size (Table 1). TEM provides the median diameter ± median absolute deviation, DLS the mean hydrodynamic diameter (Dₜ) and range, however, it cannot differentiate...
small particles if there are aggregates in the sample, explaining the lack of concordance between the average diameter provided by TEM and DLS (Table 1). The polydispersity index of tattoo inks ranges from 0.15 to 0.43; therefore, inks are not uniform in their composition.

AF4 fractionation separates samples into different sizes and MALs provides the mean gyration diameter ($D_g$) and range, the combination AF4-MALS detected a new group of NPs in the blue and green inks. Blue, violet, and green inks had a bimodal particle distribution (Table 1). Spheres have a $D_g$ and $D_h$ ($D_g/D_h$) ratio of 0.775, black inks are not spherical as 274/152 = 1.80. TEM images Figure 3F further support this structure. SP-ICP-MS provides the percentage of particles <100 nm, 67% of the NPs in the black ink were CuO whereas Figure 3 showed the composition of black inks was mainly carbon black pigment, this can be explained because SP-ICP-MS only measures metal-based NPs, like CuO, and the TEM image in Figure 3F presents unidentified particles that could be CuO.

### 1.2. Tattoo Skin Model and Biokinetics

Skin is a barrier for environmental conditions, provides sensory information and maintains homeostasis. It is formed by three layers: the epidermis, the dermis, and the hypodermis. The tattoo sensor is inserted in the dermis layer which consists of a layer of collagen, elastane filaments and glycosaminoglycans with fibroblasts, which have a dermal structure and healing function, defensive macrophages, and storage adipocytes. Skin can be used as a sensing platform for stimulus from muscles, blood vessels, free nerve endings, stratum corneum, sweat glands, and ISF. The location on the body’s anatomy, ethnicity, age, sexual hormonal influence, climate, and diet leads to varying skin features.

Porcine skin is usually used for ex vivo experiments due to its similarities (Table 2) with human skin, however, the vascularization of the human dermis is more complex, and pigs lack eccrine sweat glands. The porcine model agrees with human studies in 78%, while the rodents and in vitro studies agrees in 53% and 57%, respectively. Ex vivo experiments use lateral injections to imitate the post-healing tattoos and vertical injections to imitate the tattooing mechanism itself, showing the effect of injection depth on the sensor visibility.

Due to the close contact between ISFs, blood and lymph fluids during the invasive tattoo process, tattoo inks are regarded to be 100% bioavailable. The small and/or soluble particles in the ink formulation are absorbed into the blood or lymph vessels and are distributed into different organs where they can be stored or eliminated. The large and/or insoluble particles that cannot be absorbed and distributed are stored in the vacuoles called melanosomes from the cytoplasm of dermal macrophages. Not all ink components are inert, some can be metabolized by the cytochrome P450-dependent monoxygenase enzymes CYP or nitro-reductases; tattoo inks only undergo first phase metabolism. Sun exposure of the tattoo causes photoysis of the pigments and lightening of the tattoo. Pigments can be removed by four mechanisms: 1) by bleeding during the tattoo or healing processes, 2) by the lymphatic or blood vessel system, 3) by enzymes, 4) by light sources.

### Table 1. Size, size distribution and percentage of ink particles <100 nm; Transmission electron microscopy (TEM), dynamic light scattering (DLS), asymmetrical flow field-flow fractionation with quantification by multiangle light scattering (AF4-MALS), and single-particle inductively coupled plasma mass spectrometry (SP-ICP-MS); Ink suppliers: Millennium, Starbrite, and Intenze; Reproduced with permission. Copyright 2017, Journal of Analytical Atomic Spectrometry.

| Ink      | Diameter median TEM nm | Hydrodynamic diameter ($D_g$) DLS nm | Diameter of gyration ($D_h$) AF4-MALS nm | SP-ICP-MS |
|----------|-------------------------|-------------------------------------|------------------------------------------|-----------|
| B. Blue  | 170 ± 64                | 421 (110–980)                       | 84 (32–130)                              | CuH2Pc    |
| E. Violet| 39 ± 10                 | 31 (19–52)                          | 112 (22–200)                            | Al2O3     |
| F. Black | 19 ± 11                 | 152 (49–450)                        | 274 (42–660)                            | CuO       |
| G. Intense green | 162 ± 51 | 277 (81–1350)                   | 92 (26–112)                             | CuH2Pc    |

### Table 2. Differences in thickness, turnover cell time and in vitro model similarity between human, pig, rat, and mouse; Similarity indicates the percentage of agreement of each animal model with the human studies. Reproduced with permission. Copyright 2012, 2001, Beilstein Journal of Nanotechnology, Wound Repair Regen.

| Feature                          | Human | Pig  | Rat and mouse |
|----------------------------------|-------|------|----------------|
| Epidermis thickness (mm)         | 50–120| 30–140| 10–45          |
| Epidermis turnover (days)        | 27    | 30   | 9              |
| Stratum corneum thickness (cell layers) | 15–25 | 10–25 | 5              |
| Stratum corneum turnover (days)  | 17    | 16   | 1              |
| Model similarity (%)             | 100   | 78   | 53–57          |
1.3. Tattoo Persistence and Removal

Each time the needle penetrates, it makes a wound that activates the inflammatory process and recruits immune cells to repair the skin and clear the foreign material. The persistence of tattoos can be explained by macrophages’ physiological ability to ingest and store melanin pigment. The ink particles do not have a homogeneous size; macrophages ingest the small particles and eliminate them through the lymphatic system while larger particles remain suspended in the extracellular dermis matrix or are collected by macrophages that cannot break them down due to their size and remain stored in macrophages’ cytoplasmic vacuoles. When the macrophage dies, the pigment is released and recaptured by the new generation of macrophages (Figure 4A). Tattoo pigments can go through consecutive cycles of capture–release–recapture without any tattoo elimination. It is possible to identify the location of the pigments using flow cytometry, as un-tattooed skin scatters less light. Other theories suggest that tattoo persistence depends on macrophage longevity more than macrophage renewal since the skin’s melanophages have the slowest turnover timescales.

Diphtheria toxin (DT) selectively kills cells with the marker CD64, which includes all dermal monocyte-derived and macrophage cells. Two days after toxin exposure, the macrophages with stored tattoo ink die and release the pigment, which can be observed free in the extracellular dermis matrix (Figure 4B). 90 days after toxin exposure, the bone marrow creates monocytes to replace the dead macrophages; these cells have the same phenotype as those of green macrophages pre-DT intoxication and they recapture the ink, supporting the pigment capture–release–recapture theory. The macroscopic appearance of the tattoo does not change during this process. This model explains the difficulty of tattoo removal. Lasers heat the particle and before the particle distributes the heat around its surface, the laser repeats the pulse, and the particle deforms and breaks into smaller particles. Lasers also lyse the macrophages. The pulse duration must be briefly than the thermal calm time of the ink particles, for example, nanoseconds. The smaller pigment particles are removed by dermal macrophage cells and lymphocytes through the lymph nodes in the weeks following each laser procedure, therefore, it is important to space laser sessions at least six weeks apart. When tattoos inks are a
mixture of different colors, it causes a broad absorption spectrum making them more challenging for removal. Dark pigments are easier to remove as black absorbs all wavelengths of light. The variables to consider in a laser are wavelength, spot size, and pulse duration. Lasers used for tattoo removal are mostly commonly Nd: YAG, Ruby, and Alexandrite. Currently, lasers still cannot achieve the complete elimination of the tattoo. The combination of picosecond lasers with different wavelengths, reduces the number of sessions and provides better results, known as the rapid tattoo removal technique. The purification of traditional ink compositions to obtain specific wavelengths improve Q5 laser performance. Formulations composed of microsphere-encapsulated bioresorbable dyes, which can be removed with only one laser session, are being designed (e.g., Freedom-2 solution).

1.4. Tattoo Toxicology

Pigments are considered safe because they are inert and insoluble; nevertheless, the lack of data on inks composition and the high variability between brands make necessary the development of risks assessments to ensure safety. The legislation just considers the pigment instead of the whole formulation. When products are considered dangerous, it is communicated between the Member States and the European Commission by the European RAPEX system, the Rapid Exchange of Information System for Non-Food Product. When a patient gets a tattoo, approximately 2.5 mg cm$^{-2}$ of pigment is introduced into the skin, possibly causing mild redness symptoms, swelling, or clear exudate in the first days after getting the tattoo. Ultraviolet (UV) radiation from the sun affects causes a 20% of tattoo complaints. UV type B does not penetrate the epidermis, but type A penetrates and oxidizes the pigments increasing tattoo lightening and elimination. Cadmium and the PAAs created by exposure of azo pigments to the sun, cause the majority of the complications.

The composition of tattoo inks has been improved to reduce inflammation, for example, removing the mercury sulfide. Red inks still causing 80% of inflammatory reactions, blue and green inks also show high incidence, and black inks can produce non-allergic inflammation via aggregation of nanoparticles of carbon black. Tattoo allergies are identified by intense itching and swelling of the area, sneezing, and runny nose and eyes with an increase in lymphocyte T infiltration. There is no diagnostic reference test for tattoo allergies, the reaction has frequently a retard of weeks, months, or years after the tattoo was made. When the allergic reaction manifests, it also affects old tattoos with the same color in another area of the body. The symptoms are constant, chronic and it do not respond to topical corticoid therapy. Allergies cannot be prevented by patch testing, Bueehler test, guinea pig maximization test, or the lymph node assay because the allergen is formed over months or years within the skin, probably through metabolism, in vivo haptenisation or both, with sunlight an important precipitant factor. A specific allergy test for tattoos is urgently needed as they can also cause sensitization to other inks such as textile pigments.

Local inflammation reaction patterns include, papule-nodular, plaque-like, excessive hyperkeratotic pattern, ulcerop-necrotic, lymphatic, neuro-sensory, and scar pattern types (Figure 5A) and present different clinical and histological structures. Most papule-nodular inflammations are non-allergic reactions caused by an excess of ink (Figure 5A-i) by sarcoidosis, or by other causes such as needle trauma. Excessive hyperkeratotic inflammation, Figure 5A-ii, is indicated by heightening and thickening of the

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Figure 5. Tattoo inflammations patterns and example of pyogenic infection. A) i) Papule-nodular reaction caused by an excess of black ink with appreciable particle agglomeration, which once were nano-sized. ii) Excessive hyperkeratotic pattern. iii) Plaque-like pattern. iv) Ulcero-necrotic pattern in the red part of a tattoo. v) Lymphatic pattern, black tattoo particles located inside a lymph node, hematoxylin-eosin staining, tenfold optical magnification. Reproduced with permission. Copyright 2015, 2018, 2014, Karger Publishers, Asociación Española de Pediatría, PLOS ONE. B) Evolution of a pyogenic infection in the green ink of a tattoo. Reproduced with permission. Copyright 2015, Karger Publishers.
tattoo by vast hyperkeratosis and cornification of the surface that can end in necrosis and ulceration. Plaque-like inflammation, Figure 5A-iii, can be a result of allergy and is seen as thickness and elevation along the edge containing the problematic tattoo color. Ulcero-necrotic inflammation, Figure 5A-iv, is an intense inflammation caused by a strong allergy that develops necrosis and ulceration, and it can reach down the subcutaneous fat, muscle, or lymph nodes. Lymphatic inflammation, Figure 5A-v, is caused by an overload of ink, causing the particles to be transported to the lymph nodes, visibly coloring them. The particles block the flow of lymph, causing edema and inflammation. The pigment can also color the dermis in areas that do not belong to the tattoo design.

The tattooing process causes a release of histamine from cells due to the tattoo needle piercing the skin.[41] People with chronic autoimmune skin problems like psoriasis, lupus erythematosus, and vitiligo, having a higher chance of response when getting a tattoo being at risk for developing the Köbner phenomenon, characterized by the manifestation of the patient’s chronic illness as a response to the local lesion caused by the tattoo process.[41,53,55] The tattooing process allows microorganisms to enter the body through the needle’s penetration or healing phase[28] and can cause bacterial infections, which represent the 5% of total tattoo complications (Figure 5B).[28,41,53] Local discomfort, erythema, edema, fever, and purulence are all signs of bacterial infection in a tattoo.[53] 20% of the inks investigated in a study, including those labelled as sterile, were contaminated.[28] UV radiation produce cytotoxic reactive oxygen species in inks with genotoxic PAHs,[29,40,56] and produce carcinogenic metabolites like primary amines in yellow pigments and 3,3”-dichloro-4-aminophenyl or 3,3”-dichlorobenzidine in orange pigments.[28,43] Furthermore, tattoos hide skin areas that could be developing a neoplasm, delaying the diagnosis.[53] There is a lack of record in the epidemiological data linking tattoos and cancer; however, skin cancers in tattoos are considered coincidental. The discrepancy between the epidemiological data and the carcinogenic composition of tattoos can be explained by the ink’s single-dose exposure.[28,30,53] Studying the toxicity, bioavailability and physicochemical characteristics of traditional tattoo inks allows the design of sensors that mimic the desired features and avoid the undesired ones to maintain the advantage of a timelessness platform but also the advantage of the continuous monitoring of health biomarkers by substituting pigments with optical sensors.

2. Technologies for Tattoo Optical Biosensors

2.1. Tattoo Optical Biosensors

A biosensor is a bioanalytical platform able to change biological signals into quantitatively or qualitatively interpretable parameters.[57] They are composed of 3 elements (Figure 6A): First, a biological recognition area, which communicates with the molecule of interest, second, a transducer, which converts the biological response into a measurable signal. Finally, a processing system, which interprets the signal.[57] Sensors in medicine are used to diagnose, treat, or continuously monitor diseases. A perfect biosensor will have excellent sensitivity and selectivity for the analyte of interest, as well as sufficient temporal and concentration resolution and multiplexing capabilities.[58] Depending on the transduction method, biosensors can be optical, electrochemical, thermal, magnetic, and piezoelectric.[2,59] For example, the transducer of optical colorimetric sensors detects color changes when the analyte is present, and the color change can be correlated with the analyte concentration. Biosensing is a broad field, therefore, this article will focus on optical tattoo biosensors, where the information is obtained by modifications in the optical characteristics of the target molecule recognized by a sensor tattooed on the dermis. Photons of light interact with the sample changing its properties and modifying the initial incident light wave; a photodetector converts the changes into an electrical signal proportional to the measured analyte concentration.[57] When the light passes through the analyte (Figure 6B), it is affected by absorption, transmission, emission, elastic, or inelastic scattering from the analyte. In absorption, the light is captured and converted into heat whereas in transmission the light passes through the object changing its amplitude or intensity. Luminescent materials, like fluorescent and phosphorescent molecules, can absorb electromagnetic radiation and re-emit it in the form of visible light. In the scattering phenomena the light changes through the object. It is elastic scattering, also called Rayleigh scattering, in particles <5 nm and Mie scattering in particles >5 nm, if the light modifies its trajectory, but the energy and wavelength of the incident light does not change and the electron, after being excited, returns to its initial energy level.[40] It is inelastic scattering, also called Raman scattering, if the energy and wavelength of the incident light change and the electron, after being excited, returns to a higher or lower energy level. The energy of the
Figure 7. Example of tattoo inks’ label-based functionalization. The tattoo ink is functionalized with microparticles which produce fluorescence when the concentration of a biomarker in the interstitial fluid increases.

liberated photon differs from the energy of the incident photon and is higher in Stokes Raman scattering or lower in anti-Stokes Raman scattering.[61] The optical sensing measurement can be carried out in a solution (with particles distributed randomly) or on a planar surface (particles bound to specific locations). A greater surface-to-volume ratio is one of the benefits of sensors in suspension which allows a higher analyte binding and a better accessibility of the analyte. Simultaneously, the disadvantages are a decreased sensitivity, lower stability and reusability of the sensor, fewer multiplexing abilities, and more cytotoxic effects.[62]

Recent biosensor advancements have centered on non-invasive wearable electronic sensors placing the sensor in accessories which can be in close contact with the body for monitoring physiological parameters, like body temperature or electrophysiological signals.[63,64] A perfect wearable device would combine electrochemical performance with mechanical stress resistance while causing the least amount of disruption to the wearer’s everyday activities.[65] Wearable health technologies have the potential to early identify asymptomatic and pre-symptomatic COVID-19 infection.[66,67] However, wearable sensors require electrical power or recharging to operate and the sensing data contains environmental interferences.[9] Placing the biosensor inside the human body represents a powerful platform for continuous monitoring of biomarkers thereby providing extensive and detailed knowledge of the disease’s evolution. Tattooing the sensor into the body should be minimally invasive to allow Point-of-care (POC) use, providing high quality real-time diagnostic results within minutes. To avoid surgical procedures and reduce tissue damage or patient discomfort, it is necessary to minimize the sensor size. The sensor is injected with a tattoo needle in the dermis to be able to access the ISF, as soon as the biosensor is inserted in the skin, an unfavorable response to the foreign substance is initiated, resulting in device malfunction, poor biocompatibility, and tissue injury. This negative response can depend on the biosensor’s various properties: Form, dimension, pattern, roughness, porosity, structure, interface material/device, sterilization, time of insertion, wrapping, and metabolism.[57]

Optical biosensors are sorted into two groups based on the label’s need to generate the signal or if the analyte’s contact with the transducer produces the signal directly.[2] Besides, biosensors can be divided depending on their mechanism into, catalytic biosensors, which the biological recognition element from the sensor transforms the analyte into a metabolite (e.g., enzymes, cells, tissues) and affinity biosensors, where the analyte binds to the biological recognition element without being altered (e.g., antibodies, nucleic acids, aptamers) (Figure 6A).[57,68]

Label-based biosensing relies on direct interaction between the analyte and labelled particle through different physicochemical processes to produce a detectable signal (Figure 7). Fluorophores and phosphorescent molecules are some of the most widely used labels. Fluorescence occurs when an electron absorbs a photon from an energy source and migrates to a higher electronic state, energy is then emitted in the form of a photon when the electron falls back to the initial state thereby generating light that can be measured externally. A processing system interprets the signal providing qualitative and quantitative information about the target molecule.[62] Phosphorescent labels continue to release photons after the incident light source stops, while fluorophores need a constant energy source to glow. Fluorophores and phosphorescent labels can be proteins, peptides, small organic and inorganic compounds, synthetic oligomers, and polymers. Fluorescent proteins, such as, the yellow fluorescent protein, exhibit autofluorescence and are frequently used in biological imaging. Fluorophores and phosphorescent molecules are defined by a wavelength of maximum absorption and emission. Typically, the excitation energies are in the UV or visible spectrum while emission energies range from visible light to near-infrared. Some nanostructured semiconductor particles such as

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quantum dots (Qdots) have a very long fluorescence lifetime and a high quantum yield producing high fluorescent brightness levels. The quantum yield is the difference between the emitted and absorbed photons: the higher the quantum yield, the more substantial brightness. Qdots exhibit different emission colors depending on size and allow imaging of deep tissues using the Near-Infrared-II region. Using this spectrum, the scattering or absorption by biomolecules and autofluorescence is minimized, thereby increasing the resolution.\(^{62,69,70}\) Quantum dots have a high dark fraction as 20% to 90% of all particles never emit fluorescence. By combing QDots with other fluorophore molecules the dark fraction is reduced or even eliminated improves the photon emission’s efficiency, increasing the fluorescence lifetime. The combination of fluorophores are non-radioactive, however, improving the phosphorescence. By combing QDots with other fluorophore molecules the dark fraction is reduced or even eliminated improves the photon emission’s efficiency, increasing the fluorescence lifetime.

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Photoinduced electron-transfer sensors are triggered by analyte binding to an acceptor causing or cancelling electron transfer to a donor.\(^{62}\) When fluorophores are involved, these sensors are called fluorescence resonance electron transfer (FRET) sensors or Förster resonance energy transfer. An external excitation source provides energy to the fluorophore donor which transfers energy to the fluorophore acceptor that emits light. Due to the electron transfer, the fluorophores must be in proximity. It is advantageous to study, for example, protein interactions. Marking each protein with either the donor or acceptor fluorophore probe allows observation of whether the proteins are close enough to interact and produce fluorescence. Unlike FRET, some sensors do not require an external excitation source such as chemiluminescent resonance electron transfer (CRET) sensors and bioluminescent resonance electron transfer (BRET) sensors, reducing the unwanted absorbance of light by biological materials and the scattering of the incident light from nanomaterials, improving their sensitivity. In CRET, the analyte binds to the chemiluminescent donor which is usually an enzyme that catalyzes a reaction exciting the acceptor fluorophore and produces a signal. In BRET, the donor is a natural bioluminescent protein. The fluorophores must be in proximity. However, since BRET uses large proteins, allows higher distances between the acceptor and the donor than CRET and it is perfect for detecting in vivo protein-protein interactions. The most significant disadvantage of these biosensors is that their emission wavelengths are in the same wavelength range that biological molecules absorb light, making them not suitable for implantable biosensors now. However, recent attempts have reduced the absorption and increased the sign-to-noise ratio.\(^{62,71}\)

Label-free biosensors are ideal for molecules which are complicated to label or have no adequate label identified. Moreover, they have a reduced analysis time, consumption of organic solvents, size, low cost, high sensitivity and they can quantify molecules in real-time.\(^{72}\) The interaction between the analyte and the transducer produces the signal directly. Surface plasmon resonance (SPR) is measured by direct stimulation of low molecular weight molecules incident light that is polarized at a certain angle, known as the resonance angle. The interaction of the light with the sample generates surface plasmons on the surface of a conducting material, like a metal, sandwiched between two media, like glass and liquid. The transducer can recognize a change in the wavelength and the resonance angle when the plasmons are generated. This change is found to be proportional to the mass of analyte at the surface and therefore the concentration of the analyte in solution.\(^{2,62}\) SPR biosensors are able to detect, monitor, and quantify biomarkers, detect molecular interactions, and time-dependent binding interactions.\(^{73}\) Localized surface plasmon resonance (LSPR) is the resulting effect of limit SPR in a metal nanoparticle enhancing the electric field close to the surface of the particle. The LSP is concentrated in a tiny area at the nanoparticle surface, and the resonance may be altered by modifying the physicochemical properties of the nanoparticle. Using nanostructured materials instead of films enhance the SPR signal and enable the detection of hard-to-find biomarkers; because of their chemical stability, gold nanoparticles are widely employed even though silver ones provide better LSPR bands. SPR and LSPR methods have the possibility to be employed in a variety of biosensing applications, however, many and important challenges remain before they can be efficiently used in practice including their sensitivity, specificity, and reproducibility.\(^{74-77}\)

Surface-enhanced Raman spectroscopy (SERS) allows ultrasensitive imaging and sensing to detect inelastic Raman scattering.\(^{62}\) The Raman spectrum is characteristic for each molecule and allows a qualitative and quantitative analysis of a mixed sample.\(^{2,61}\) An example of functionalization of tattoos with label free sensing techniques is developed by the group of Wang et al., with a SERS tattoo for sensing of food toxins.\(^{12}\) The sensor comprises of a gold coated tattoo that can transmit an efficient plasmonic pattern to various surfaces for target sensing, for example for the detection of a fungicide (thiabendazole) used in orange preservation and packaging. The results provide a limit of analyte detection at 0.1 \(\mu\)M, below the EU specified maximum value, however, the sensitivity of these tattoos is lower than other alternatives. Monitoring biomarkers in the ISF by surface-enhanced Raman scattering is also possible, SERS tattoos were in vivo tested in pigs and the results showed the capabilities of these tattoos for the continuous monitoring of nucleic acids.\(^{10}\) SPR and SERS are the most common optical biosensor transducers for viral detection.\(^{78}\) The ultrasensitive and specific potential for biomarker detection with Raman spectroscopy has piqued interest for the detection of SARS-CoV-2.\(^{79,80}\) SERS can distinguish between viruses, viral strains, and viruses with gene deletions in biological medium based on their spectral differences.\(^{81}\) it is a potent advantage and opportunity for tattoo functionalization.

### 2.2. Interstitial Fluid as a Substitute for Blood Biosensing

Traditional tattoo inks can be replaced with biosensors that can inform about different biomarkers in the dermal ISF, the sensor is injected with a needle in the dermis to be able to access the dermal ISF.\(^{9}\) Extravasation of plasma from capillaries results in the formation of the ISF, it is composed of composed of water with polysaccharides, lipids, proteins, and electrolytes, such as sodium (Na\(^{+}\)), chloride (Cl\(^{-}\)), bicarbonate (HCO\(_3\)^{-}\)), potassium (K\(^{+}\)), calcium (Ca\(^{2+}\)), and magnesium (Mg\(^{2+}\))\(^{9}\) and it is a medium via which nutrients and undesirable substances are exchanged between blood arteries and cells.\(^{82}\)

The flow between the blood capillaries, the interstitial space, and the lymphatic capillaries is regulated by the hydrostatic and...
are potent and powerful pH buffers maintaining a constant blood pH.

The composition in blood and ISF is comparable except for the reduction of calcium concentration by half and protein concentration by one third in ISF compared to plasma. Proteins, due to their high molecular weight, barely diffuse into the ISF. The oncotic pressure of the ISF must always be lower than in the capillary blood to allow passive diffusion of the biomarkers into the tissue. Calcium can be found in plasma as free calcium ions, as inorganic complexes or as calcium bound to proteins, explaining why calcium concentrations are lower in ISF. Although the pH range differences are not very significant (7.35–7.4 for plasma and 7.1 and 7.4 for ISF), the average value is more unstable in the ISF as proteins such as hemoglobin or albumin are absent and powerful pH buffers maintaining a constant blood pH.

Glucose in ISF is a widely studied biomarker. As shown in Table 3, plasma and ISF concentrations are comparable, 5.05 and 5.42 mmol L\(^{-1}\), respectively. Average fasting plasma glucose levels continue to increase by 0.07 mmol L\(^{-1}\) in women and 0.09 mmol L\(^{-1}\) in men per decade since 1980, it is essential to have reliable and safe devices that continuously control blood sugar levels in real-time and tattooed biosensors are promising candidates. Continuous glucose monitoring (CGM) devices meet this need, however, the average error percentage for CGM devices runs at around 15%. The calibration of the measuring device is the primary source of sensor error as it can be performed during rapid plasma glucose changes.

The best method to monitor blood biomarkers is measuring blood directly; however, this practice is limited by the requirement of a specialist, the impossibility of continuous monitoring, and discomfort associated with drawing blood. Urine and saliva are easier to measure compared to ISF, although they have limited biomarkers and unstable concentrations.

Notes: The mean value and standard deviation are shown in units according to the International System of Units.
a comfortable alternative to blood sampling that allows continuous monitoring and multiplexing capacities for the control of more than one biomarker, especially convenient in metabolic diseases such as diabetes where the illness decompensates more than one physiological parameter, for example glucose and pH. The “Dermal Abyss” project aims to create a continuous glucose monitoring platform using an enzymatic biosensor made of glucose oxidase, peroxidase, and potassium iodide. When interstitial glucose levels increase the biomarker is metabolized by the enzyme glucose-oxidase creating gluconolactone and hydrogen peroxide. The enzyme peroxidase catalyzes the production of hydrogen peroxide and reacts with the potassium iodide producing iodine. The iodine produces a measurable color signal at 680 nm which relates the iodine values indirectly to the glucose concentration. The tattooed biosensors can send the measured concentrations to a smartphone for interpretation and store the data. Other enzymatic glucose biosensors use both enzymes but substitute potassium iodide with 3,3”,5,5”-tetramethylbenzidine. The hydrogen peroxide formed reacts with the 3,3”,5,5”-tetramethylbenzidine under peroxide catalysis producing a color change from blue to green with an absorbance peak at 680 nm. This allows to control glucose changes from concentrations of 2 mmol L⁻¹ up to 50 mmol L⁻¹. Despite promising results, both projects require more permanence, reversibility, and color range. The dependence on the use of enzymes has a negative impact on the reversibility of the sensor; their substitution for diboronic acid provides reversible glucose responsiveness. Shibata et al. design a glucose sensor (Figure 9A) which consist of two diboronic acids groups which act as the recognition site for the glucose and one anthracene molecule which is the fluorophore. This component has been tested in injectable hydrogel microbeads which can be introduced in traditional tattoo inks for in vivo CGM by fluorescence (Figure 9B) and the results proved the reversibility of the beads and a successful control of glucose monitoring (Figure 9C), however, due to their small size (130 μm), the microbeads dispersed from the implantation site one month after being injected. Increasing the size (1000 μm), changing the shape to fibers, and adding polyethylene glycol to the formulation improved the biocompatibility and increased the sensor’s response time to glucose (up to 140 days), but still maintaining some challenges related with the use of fluorophores like the photobleaching, calibration, or the potential risks of excessive skin irradiation. Sensing the ISF goes beyond the glucose biomarker. The “Dermal Abyss” project also offers sensors capabilities for other clinical biomarkers, like the monitorization of sodium by using the fluorophore diaza-15-crown-5 to control dehydration states caused by loss of electrolytes or hypertension caused by hypernatremia. Other example is the monitorization of pH by the fluorophore seminaphthorhodafluor or by the anthocyanin which...
changes color from red in acidosis to blue in alkalosis staying violet at neutral pH. Other popular targets for tattoo interstitial biosensing include potassium, oxygen, cortisol, lactate, and albumin concentrations (Figure 10A, B).

An important advantage in optical biosensing is the simplicity in data collection. The dermal sensors quantitative records can be collected by a smartphone camera (Figure 11A). In the case of multiplex tattoos, each sensor must be photographed separately (Figure 11B). A mobile application allows the interpretation and storage of the measured data, improving the communication between doctor and patient to enable individualized medicine as the data could be sent to the health center (Figure 11C). Portable compatible optical readout devices may be connected to the dermal tattoo sensor to improve the accuracy and stability of the electrolyte levels. Wearable devices with optical capabilities can also be employed in combination with optical functionalized tattoos for sensing. Since alcohol abuse leads to accidents and health problems, temporary-tattoo sensors can also monitor alcohol concentration (Kim et al., 2016) and raise awareness of responsible consumption. The functionalization of tattoos goes beyond the sensing biomolecules, an example is the use of tattoos for microballistic transdermal doxorubicin drug delivery by ultrasound, or the release of anti-inflammatory drug encapsulated in alginate microspheres. This epidermal tattoo-patch enhances skin penetration of the drug avoiding the use of needles.

3. Future Perspectives and Challenges

There is a potential future use of tattoos for DNA vaccination since tattoos provide higher peptide-specific immune responses than intramuscular needle injections. The dermis contains plenty of antigen-presenting cells, which create an efficient antigen-specific immune response. Moreover, tattoos are useful for vaccination with peptides who aggregates easily, and it allows to remove adjuvants on the vaccine composition reducing size effects. The tattoo biosensing functionalization might play a major role in combating viral pandemics with ongoing research and improvement. Other different projects demonstrating the future capabilities of tattoo functionalization beyond ISF biosensing have been initiated to create an interactive platform with our skin where biosensors are no longer an external device, they are an extension of our body providing new capabilities. Butterfield et al. demonstrate the use of UV-sensitive photochromic nano-capsule tattoo ink to indicate overexposure of UV light (Figure 12A–E), and the need for sun protection, known as...
Figure 11. Reading process of a multiplexed tattooed optical sensor. A) Quantitative readouts using a smartphone camera at 5.0 mm normal incidence to the detection area. Reproduced with permission.[5] Copyright 2018, Wiley-VCH GmbH. B) Multiplexing capacities of tattoo functionalization with fluorescent sensors: seminaphthorhodafluor pH-sensor, diaza-15-crown-5 sodium-sensor, and benzofuran isophthalate tetraammonium salt potassium-sensor. Tattoo under normal light and LED excitation. Scale bar = 5 mm. C) Screenshots of the smartphone apps used for rapid detection and point-of-care diagnosis. The smartphone app shows the selection of the sensor type (left), imaging of the test samples (middle), the quantitative and diagnostic results are displayed to the user (right). Reproduced with permission.[4] Copyright 2020, Sensors and Actuators B: Chemical.

“solar freckles.”[18] The capsules are reversible since their color disappears rapidly after application of topical sunscreen. The major cause of skin cancer is UV exposure, these tattoos would increase preventative behaviors providing a visual reminder to protect the skin and would reduce the incidence of this pathology. These tattoos are semi-permanent and are long lasting, remaining functional for months to years. In addition to being incorporated into the skin as tattoos, UV-sensitive sensors can be used in skin patches, contact lenses, textiles, or wearable devices.[120] UV-fluorescent tattoos can also be used for identifying biopsy sites at the time of surgery.[121]

Temporary cell-based tattoos have also shown a lot of interest. One example is provided by Tastanova et al., who developed a synthetic cellular biomedical tattoo for detecting cancer-related hypercalcemia. The cells expressed calcium receptors which reacted when calcium increased in blood and produced black pigment melanin. The sensing component was a calcium-sensing receptor, the visualization component was the development of a visible tattoo by the accumulation of melanin produced by the oxidation of tyrosine by the enzyme tyrosinase in the melanosome of melanocytes of a transgenic HEK_{Tattoo} (human embryonic kidney) cell line (Figure 13). The HEK_{Tattoo} cells were microencapsulating in alginate—PLL (poly-L-lysine)—alginate beads. The system was confirmed in wild-type mice tattooed with the encapsulated engineered cells, there was appearance of tattoos in mice with hypercalcemia breast and colon adenocarcinoma cells while there was no tattoos in ones with normocalcemic tumor cells, validating the use of cell-based tattoos for detect hypercalcemia associated with cancer and its potential for future cancer management.[122] Another example for the possibilities that the combination of cells and tattoos offers is the “Living Tattoos” project of the Massachusetts Institute of Technology, which consists of a temporary skin-patch tattoo, composed of bacterial cells mixed with hydrogels and nutrients. The cells can act as a chemical optical biosensor for environmental components, contaminants, as well as, for changes in pH or temperature by emitting fluorescence. Each cell in the tattoo would perform a simple computational operation and different cell types can interact between them in the same tattoo to achieve complex logic operations. The main challenges are the precision to incorporate the cells into the hydrogel matrix and...
to maintain the viability and responsiveness of the cells.\textsuperscript{[11]} Many new high-stretchable functional materials have been proposed as candidates for use in temporal tattoos, most of them come from the use of specialized synthetic materials/composites or heterogeneous collections of material micro/nanostructures.\textsuperscript{[63]} This review article is focused on tattoos made with implantable optical biosensors for monitoring biomarkers in ISF. However, it should be mentioned that there are other types of tattoo sensors for plenty different future applications, highlighting electrochemical sensors in temporary skin-patch tattoos. The use of graphene in electronic tattoos sensors act as commercially available wearable health and fitness trackers; patients’ electrocardiogram, electromyogram, electroencephalogram, temperature, and hydration are all effectively controlled by these tattoos. Graphene is an electrically conductive material, optically transparent, robust, electrochemically stable, and biocompatible, however, it is too large to be used as a tattoo ink, so it has to be transferred on human skin like a temporary tattoo.\textsuperscript{[16,123,124]} Another example of the advantages of using 2D materials for temporal tattoo biosensing in electronic tattoos sensors is the use of carbides and nitrides called MXenes which are nanomaterials that successfully measured pulse, respiration rate, and surface electromyography.\textsuperscript{[125]} The advantages of these tattoos include excellent mechanical flexibility, small size, comfort, and high adhesion to the skin. The “Duoskin” project introduces three different types of tattoos functionalized with the use of gold leaves.\textsuperscript{[19]} The first type is touch sensitive input tattoos, allowing the skin itself to act as a control unit, the second type display data, allowing the monitoring of body parameters, such as temperature changes. The last type are communicative tattoos, which convert the skin into an interactive platform which wirelessly exchanges information with other devices.\textsuperscript{[19]} With this type of temporary electronic tattoos sensors, information such as identity documents or credit card details would be incorporated into our skin. The functionalization of tattoos seems to be a very promising field for plenty different future applications.

Optical biosensors in the last decade have developed extensively in different fields, including therapeutics, toxicology, environment, military, and safety, but lot remains to be done before traditional methods can be fully replaced.\textsuperscript{[2]} The examples above show how tattoo sensors have progressed over the last decade and their future applications. However, several challenges arise when designing a tattoo sensor for ISF biosensing, some related to the biological sensing platform and others to the optical sensors. The main biological challenges include the time delay that exists to reach equilibrium between the concentrations of the biomarker of interest in blood and ISF.\textsuperscript{[3]} As it has been shown for glucose (Table 3), the lag time could be up to 8 min. This challenge can be reduced by using mathematical models that algorithmically correct the delay by considering all the parameters that influence the regulation of the specific biomarker;\textsuperscript{[110]} it is crucial to study the physiological regulation of the biomarker for which the tattoo is to be designed. An advantage and disadvantage for ISF biosensing is the inherent composition of this fluid, it is a complex matrix with many components which allows multiplexing capacities for the sensor,\textsuperscript{[7]} making it suitable for monitor more than one biomarker and provide a complete control of the patient’s pathology, but at the same time it hinders the specificity of the sensor as there are very similar species in the same medium. Also other physiological fluids, like sweat can affect to the stability of the dermal sensor and can be prevented with sensor coatings.\textsuperscript{[126]} Biofouling is the accumulation of proteins, cells and other biological materials on a surface, it is one of the main challenges for in vivo biosensors.\textsuperscript{[127]} It can be reduced by modifying the sensor surface to prevent non-specific interactions or by antibiofouling membranes.\textsuperscript{[17]} Physiological components can also produce optical signals that can interfere with the measurement of the biomarker of interest.\textsuperscript{[72,128]} is extremely important to achieve high signal-to-background ratios by using high-precision devices.\textsuperscript{[62,21]} The influence of the
anatomical location of the tattoo, the tattoo implantation depth and the skin differences between ethnicity, age, sexual hormonal impact, climate, and diet should be studied. The skin is subjected to an almost constant mechanical stress which can affect sensor’s performance. 3D printing allows the creation of temporal tattoos with highly stretchable sensors in hydrogels.

Diffusion of the sensor from the tattoo site into the ISF is a challenge that must be controlled by modifying the sensor size, but is a difficult challenge since they should be small enough to avoid surgical procedures but should be large enough to be retained in the desired area, tattoo biosensors should mimic in size the traditional tattoo pigments. The dimension of the implanted sensor also affects the degree of tissue damage. An example of the importance of size is the miniaturization of a CGM sensor using microbeads to coat and reduce the sensor’s size.

The microbeads, <1 μm, were inserted into the skin using a needle, however, they were not suitable for long-term monitoring in vivo as they dispersed from the implantation site and permeated the cell-membrane proving challenging to remove. Another attempt to formulate the microbeads as fibers of 1000 μm in diameter for long-term application, however, the fibers needed to be removed after three months and local inflammation reactions were registered. Biocompatibility encompasses the physiological reaction to the implanted sensor as well as the technological reaction in the sensor. To improve biocompatibility, biomimetic materials are used, for example soft and flexible polymeric hydrogels, or porous silicones which are natural components in our body found in collagen fibers and bones, when the sensor degrades, the metabolites formed are non-toxic and natural orthosilicic acids, which are safely eliminated from the body.

The use of coatings with self-healing properties that actively respond to the in vivo environment enhances the biocompatibility of the sensor. A novel strategy to improve the biocompatibility of tattooed sensors for monitoring glucose in the ISF was the introduction of anti-inflammatory-drug-loaded alginate microspheres into the tattoo itself, creating a combined smart tattoo that releases drug but also monitors biomarkers. The drug was successfully and completely released microspheres over a period of 3–4 weeks improving not only the biocompatibility of the sensor, also its in vivo functionality.

Tattooed enzymatic biosensors exhibited excellent capacities for interstitial biomarker monitoring. However, their main challenges include a short-duration functionality, a lack in long-term reversibility, dependence on oxygen levels, low precision, low stability, or loss of activity because of immobilization; the variety of colors and intensities of the contemporary enzymatic biosensors should be increased to allow higher-resolution signals. Other label-based optical sensors based on fluorescence are affected by photobleaching, light scattering, cytotoxicity, potential health risks of excessive skin UV irradiation, and weak fluorescence signal; pulsed excitation system is required to minimize photobleaching. The accurate of the sensor redouts can be improved using portable and customizable optical readout devices. Some sensors, like QDots, contain heavy metals making their long-term use impossible in vivo due to the risk of toxicity from leakage of components. Encapsulation of QDots in nanoparticles is a powerful solution to ensure patient safety, also providing beneficial properties to the sensor like enhancing its half-life. Label-free techniques have not yet been used for continuous in vivo monitoring as the sensor surface, miniaturization, and multiplexing viability must be improved.

The main challenge of label-free optical biosensors is their low sensitivity and specificity for the detection of the biomarker of interest in a complex sample, like ISF. Despite the difficulties described, continuous biomarker monitoring by minimally invasive and long-lasting methods is an advantageous development toward personalized medicine. Secondary diseases from uncontrolled biomarkers cause enormous societal and economic costs; effective and continuous health monitoring with multiplexing capacities can prevent them, improve the quality of life of patients and enables individualized medication. Since the ISF is rich in biomarkers, the designed dermal tattoo sensors can be employed to real-time and long-term monitoring of biomarkers for multiplexed POC diagnostic devices.

4. Conclusions

In addition to aesthetics, tattoos could be platforms for diagnosing or health monitoring. Their permanence under the skin
provides a useful advantage for substituting pigments with implantable biosensors. The sensor is introduced into the dermis with a tattoo needle to monitor biomarkers in the ISF, providing high-quality, real-time diagnostic results of ISF composition within minutes, which will be used as POC devices. In this article, an analysis of the functionalization of tattoos for optical biosensing in ISF has been made discussing the components and mechanisms involved. From the analysis of several tattoo brands, it can be concluded that there is a high variability in the ink composition between different manufacturers due to the lack of pharmaceutical regulation. Tattoo inks are a thermodynamically unstable dispersions of insoluble pigments in a solvent, they can contain allergic metals, cytotoxic nanoparticles, or toxic metals above the safety limit. Long-term tattoo persistence is well understood by macrophage capture–release–recapture renewal theory. Pigments are considered safe because they are inert and insoluble; nevertheless, there is a necessity to develop risk assessments as part of the injected tattoo ink is also absorbed into the blood and distributed to other body areas. Tattoo inflammations are the most reported complications and allergy inflammations cannot be prevented because they are produced in vivo haptenisation; the delay in the onset of symptoms also explains the lack of records of tattoo complications. ISF can substitute blood as a sensing platform since the composition is similar except for calcium and proteins, which are higher in the blood. It also provides new clinically valuable biomarkers. Placing the biosensor inside the human body represents a powerful platform for continuous monitoring of biomarkers reducing environmental interferences. Using optical biosensors avoid the necessity of electrical power nor recharging. Some examples of tattoo biosensing for ISF biomarkers, the different types of optical biosensors and their main challenges and future perspectives are discussed. The potential opportunities offered by the functionalization of tattoos cover different fields and seems limitless. Functionalized tattoos for interstitial biosensing provide effective way to monitor and record the evolution of a patient’s disease with the capability of measure more than one biomarker within the same tattoo. Preventing future complications means an improvement in personal quality of life and economic savings for society. Secondary diseases from uncontrolled biomarkers cause enormous societal and economic costs; effective and continuous health monitoring with multiplexing capacities can prevent them, improve the quality of life of patients, and enable personalized therapeutic decisions.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

M.D.P.: Conceptualization, writing original draft, review and editing, visualization, funding acquisition. Y.H.: Project administration, writing—review and editing. Y.E.: Writing—review and editing, funding acquisition. N.J.: Conceptualization, writing—review and editing, funding acquisition. A.K.Y.: Conceptualization, resources, supervision.

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[1] P. Minghetti, U. M. Musazzri, R. Dorati, P. Rocco, Sci. Total Environ. 2019, 651, 634.
[2] R. Damborský, J. Švitel, J. Katrlík, Essays Biochem. 2016, 60, 91.
[3] H. Shibata, Y. J. Heo, T. Okitsu, Y. Matsuenga, T. Kawanishi, S. Takeuchi, Proc. Natl. Acad. Sci. USA 2010, 107.
[4] N. Jiang, A. K. Yetisen, N. Linhart, K. Flisikowski, J. Dong, X. Dong, H. Butt, M. Jakobi, A. Schnieke, A. W. Koch, Sens. Actuators, B 2020, 320, 128378.
[5] A. K. Yetisen, R. Moreddu, S. Seifi, N. Jiang, K. Vega, X. Dong, J. Dong, H. Butt, M. Jakobi, M. Elsner, A. W. Koch, Angew. Chem., Int. Ed. 2019, 58, 10506.
[6] C. Samson, A. Koh, Front. Bioeng. Biotechnol. 2020, 8, 1037.
[7] A. Kaushik, A. Vasudev, S. K. Arya, S. K. Pasha, S. Bhansali, Biosens. Bioelectron. 2014, 53, 499.
[8] W. Jia, A. J. Bandodkar, G. Valdés-Ramírez, J. R. Windmiller, Z. Yang, J. Ramirez, G. Chan, J. Wang, Anal. Chem. 2013, 85, 6553.
[9] K. Vega, N. Jiang, X. Liu, V. Kan, N. Barry, P. Maes, A. Yetisen, J. Paradiso, ACM International Symposium on Wearable Computers 2017, 1, 138.
[10] H.-N. Wang, J. Register, A. Fales, N. Gandra, P. Strobbia, E. Cho, A. Boico, C. Palmer, B. Klitzman, A. W. Koch, Sens. Actuators, B 2019, 261, 218.
[11] A. Zorman, C. K. Dhillon, A. W. Koch, Sens. Actuators, B 2020, 6553.
[12] F. Soto, R. K. Mishra, R. Chrostowski, A. Martin, J. Wang, Adv. Mater. Technol. 2017, 2, 1700210.
[13] J. H. van den Berg, K. Oosterhuis, T. N. Schumacher, J. B. Haanen, A. D. Bins, Methods Mol. Biol. 2014, 1174, 131.
[14] J. Kim, I. Jeerapan, S. Imani, T. N. Cho, A. Bandodkar, S. Cinti, P. P. Mercier, J. Wang, ACS Sens. 2016, 7, 1011.
[15] D. Kireev, S. K. Ameri, A. Nederveld, J. Kampfe, H. Jang, N. Lu, D. Akinwande, Nat. Protoc. 2021, 6, 2395.
[16] D. R. Seshadri, R. T. Li, J. E. Voos, J. R. Rowbottom, C. M. Alves, C. A. Zorman, C. K. Drummond, npj Digital Med. 2019, 2, 72.
[17] J. L. Butterfield, S. P. Keyser, K. V. Dikshit, H. Kwon, M. I. Koster, C. J. Burns, ACS Nano 2020, 14, 13619.
[18] H.-L. Kao, C. Holz, A. Roseway, A. Calvo, C. Schmandt, ACM International Symposium on Wearable Computers 2016, 1, 16.
[19] K. Sperry, Am. J. Forensic Med. Pathol. 1991, 12, 313.
[20] F. Rosenkilde, Curr. Probl. Dermatol. 2015, 48, 21.
Marta Lucía Domínguez Pazos is currently working as a Researcher at the NanoBiosensors and Bioanalytical Applications Group, Institut Català de Nanociència i Nanotecnologia in Barcelona and she is a Ph.D. candidate in this institution for the biochemistry, molecular biology and biomedicine program from the Universitat Autònoma de Barcelona. She completed her bachelor’s degree in pharmacy at the University of Santiago de Compostela (2019) and her M.Sc. in pharmaceutical sciences at the University of Copenhagen. Her master’s thesis focused on the functionalization of tattoos with optical biosensors for monitoring blood sugar levels at the Yetisen Research Group, Imperial College London (2021).

Yubing Hu is a research associate and assistant supervisor in the Department of Chemical Engineering at Imperial College London. Dr. Hu received a bachelor’s degree from Zhejiang University in 2016 and earned a Ph.D. degree from the Hong Kong University of Science and Technology in 2020. Her Ph.D. study focused on the development of fluorescent polymer materials for advanced sensing and imaging applications. Her current research aims to develop a variety of optical biosensors for wearable diagnostic devices. Her research works have been published in Advanced Materials, Advanced Functional Materials, and CCS Chemistry.

Yuval Elani is a UKRI Future Leaders Fellow and Lecturer at the Chemical Engineering Department at Imperial College London. His group works on biomimetic technologies, microfluidics, and synthetic biology. Yuval previously held EPSRC and Imperial College Research Fellowships at Imperial Chemistry. Prior to joining Imperial, Yuval was at Cambridge University, where he studied natural sciences. Yuval has been awarded several prizes for academic excellence, including the Royal Society of Chemistry Felix Franks Medal for Biotechnology, Rita and John Cornforth Award, Roscoe Medal, and the Imperial President’s Medal.

Kathryn Browning is an assistant professor in the LEO Foundation Center for Cutaneous Drug Delivery in the Department of Pharmacy at the University of Copenhagen, Denmark. She received her Ph.D. in physical chemistry from the University of Cambridge, UK. Following this, she worked as a postdoctoral researcher in Uppsala University, Sweden, using neutron scattering to study lipid exchange and removal in atherosclerotic lipoproteins. Her current research focuses on drug delivery through the intact stratum corneum.
**Nan Jiang** earned her Ph.D. degree from Wuhan University of Technology. After her Ph.D. study, she worked as a postdoctoral fellow and a research associate at Harvard University and Imperial College London. She is currently working as a faculty member at Sichuan University. Her research is aimed at optical biosensors and microfluidic devices. She has 27 peer-reviewed papers as first author (co-first author) and corresponding author. Some important works have been published on leading journals such as *Advanced Materials, Advanced Functional Materials, Energy & Environmental Science*, and *ACS Nano*. Some works have been selected as “Cover paper.”

**Ali K. Yetisen** is a Senior Lecturer and Associate Professor in the Department of Chemical Engineering at Imperial College London. He was previously a Tosteson fellow at Harvard University. He holds a Ph.D. degree in Chemical Engineering and Biotechnology from the University of Cambridge. He has been awarded several international prizes including IChemE Nicklin Medal, Birmingham Fellowship, MGH ECOR Award, Humboldt Research Fellowship Award, Carl Friedrich von Siemens Fellowship Award, and a Fellowship of the Royal Society of Chemistry.