Received: 29 October 2021 | Revised: 26 January 2022 | Accepted: 6 February 2022

DOI: 10.1002/ppap.202100188

BRIEF COMMUNICATION

PLASMA PROCESSES AND POLYMERS

Can we remove tattoos with non-thermal atmospheric plasma?

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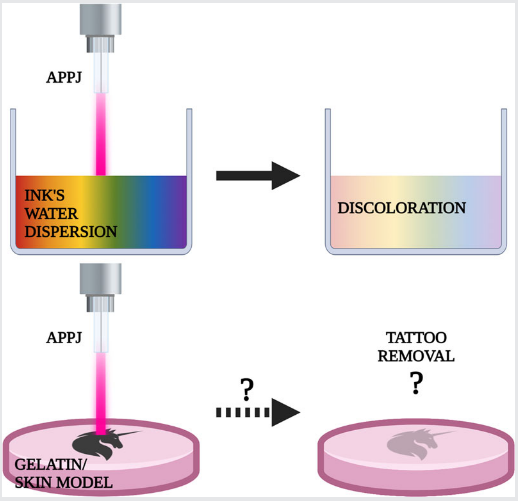
Funding information
H2020 European Research Council, Grant/Award Number: 714793; Secretaría de Estado de Investigación, Desarrollo e Innovación, Grant/Award Number: PID2019-103892RB-I00/AEI/10.13039/501100011033

Abstract
Current methods for tattoo removal are long, costly and have drawbacks such as scarring among others. Looking for alternatives, here we assess the feasibility of non-thermal plasma (NTP) as a standalone method for the removal of tattoos. We report the results of atmospheric pressure plasma jet treatment of real tattoo inks suspensions in water and in gelatin (liquid or as a solid film), selected as a model of skin. Analysis of the residual color and the temperature effect in all the samples after plasma treatment reveals significant differences between water and the skin model. Kinetic considerations and the extension of our results to more realistic scenarios allow us to conclude that NTP cannot compete with the current laser technology in a real application.



KEYWORDS
cold plasma, decomposition, gelatin, ink, skin

1 | INTRODUCTION

Non-thermal plasma (NTP) technology has opened up many opportunities in dermatology as promising effects have been reported in wound healing, treatment of psoriasis, atopic dermatitis and skin cancer.[1–3] NTP has also been successfully reported as an advanced oxidation process capable of oxidizing and decomposing persistent organic pollutants like pigments and dyes from wastewater.[4,5] Combining these two assets, it seems natural to inquire if NTP could also decompose artificial pigments in the skin.

In other words, is it possible to remove tattoos by treating them with NTP?

The tattoo removal industry is increasingly profitable as many people own one or more tattoos (12% of Europeans and up to 24% of US citizens are estimated to be tattooed),[6] and according to a 2014 review, up to 50% of the tattooed individuals regret their tattoos to some extent.[7] At present, the most widely used methods for tattoos removal are Q-switched lasers that are mainly based on the principle of selective photothermolysis.[8] Photons generated by lasers with different wavelengths are selectively absorbed by tattoo pigment particles, according to their specific absorption properties, causing rapid local heating and fragmentation. Then, the remaining small pigment particles and any decomposition products generated during the treatment are removed from the skin through the vascular system. This system is not yet ideal as its cost is very high, patients may experience pain and require multiple treatments. Moreover, some patients can experience permanent changes in skin pigmentation, textural changes, scarring and allergic reactions, so the quest for alternative treatments is open.

NTP could be a less selective (wide range of UV–VIS radiation) but milder treatment, possibly with less thermal effects. Moreover, NTP generates short-lived reactive chemical species (usually reactive oxygen and nitrogen species) and UV radiation that are able to oxidize and therefore decompose organic and inorganic molecules. There are very few reports of studies that have investigated the feasibility of NTP-assisted tattoo removal. Cukur and Ercan tested NTP in the treatment of diluted tattoo inks dispersed in agarose gel obtaining a measurable fading effect.[9] Few patent applications have been published in the last 15 years based on combinations of NTP and mechanical treatment, but these processes did not reach the market yet.[10,11] Thus, to the best of our knowledge, no self-standing NTP technique is currently available for tattoos removal.

The aim of this communication is to investigate the feasibility of NTP-assisted tattoo removal. To do this, we used a helium NTP jet in the open air to treat tattoo inks dispersed in phosphate buffer and in gelatin (in solution or in solid films), as a model of the skin, and we analyzed the treated samples to understand the kinetics of pigments removal and the temperature effect of the treatment on the samples, to discuss on the possible translation of this therapy or not.

2 | EXPERIMENTAL SECTION

2.1 | Materials

Gelatin from bovine skin (gel strength ~225 g Bloom, Type B; Sigma-Aldrich). Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$, >98.0%; PanReac). Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, >98.0% PanReac). Ultrapure water was obtained by filtration of deionized water using a 0.22-µm pore size MILLEXGP filter unit (Merck Millipore). Compressed helium (>99.998%, maximum impurities of O_2 and H_2O 5 ppmv; Praxair). Four different colors (red, blue, yellow, and black) of tattoo inks (commercial names: Ultra Red, Blue, Golden Yellow, and Tribal) were purchased from Viking-Ink (https://vikinginkgroup.com/). According to the supplier, they contain distilled water, witch hazel, isopropanol, and pigment(s). The red ink (Ultra Red) also contains glycerin. The specific pigment(s) of each ink is not disclosed and neither is the concentration of all the components. The pigments are not water-soluble.[12]

2.2 | Preparation of the samples

All solutions and suspensions used in this study were prepared in phosphate buffer (PB) 50 mM pH 7 to ensure the stability of the pH during plasma treatments. One thousand parts per million volume (0.1% vol/vol) stock suspensions of tattoo inks in PB were obtained by diluting pure inks 1:1000 in graduated flasks. Working suspensions were prepared by further dilutions with PB.

Liquid gelatin samples containing tattoo inks were prepared by diluting pure inks 1:1000 using a 0.02 g/mL gelatin solution; the final inks concentration was 1000 ppmv. Diluted samples were prepared by mixing these suspensions with the appropriate amount of 0.02 g/ml gelatin solution without inks.

All liquid samples were homogenized after preparation and before each use using a Vortex Mixer (Velp Scientifica) for 2 min at 16 rpm.

Solid gelatin disks (35 mm diameter and 2 mm thickness) containing tattoo inks were prepared as follows: first pure inks were diluted 1:1000 in a 0.15 g/mL gelatin solution (final inks concentration 1000 ppmv); then, 2 mL aliquots were transferred in a 12-well plate and stored at 4°C for at least one hour to solidify.

All the samples containing gelatin were prepared no longer than 2 days before use and stored at 4°C tightly sealed with laboratory film.

2.3 | Plasma source and treatments

The NTP source used in this study is an atmospheric pressure plasma jet (APPJ), operating with helium.[13,14] A Bronkhorst Mass-View flow controller was used to set the helium flow rate. Experiments were performed in the open air, at room temperature and with an average relative humidity of 70%. More details and a picture can be found in Figure S1.

All the treatments, if not otherwise stated, were performed using the following conditions: helium flow rate 1.00 L/min, the distance between plasma nozzle and target 10 mm. A sample volume of 1.00 mL in a 24-well plate for liquid samples and 2.00 mL in a 6-well plate for solid gelatin disks. The helium flow was started 15–20 min before each treatment to ensure a good purging of the system. The plasma discharge was started at least 5–10 min before the treatment to let it stabilize. Each treatment was done in triplicate. Water evaporation during the treatment of liquid samples was quantified in a previous work under the same experimental conditions reported here[15] and taken into account in subsequent analyses.

Two treatment modes were used: point treatment and area treatment. In point treatment mode, the APPJ was fixed in the same position during the whole duration of the treatment. Therefore, the jet impinged in the same point of the target for all the treatment duration. This mode was used for liquid samples while solid gelatin films underwent both point or area treatment. Treatment time was varied between 30 and 900 s. In area treatment mode a $15.0 \times 15.0\ mm^2$ area of the solid gelatin disks was treated by moving the source following a snake-shaped pattern during the treatment using a 3D robot (High-Z S400/CNC Router, CNC-Step). The total duration of area treatment was 600 s with 12.8 mm/s speed.

2.4 | Analyses

The temperature of the samples, before, during and after the treatments were measured using a Thermal Camera GTC 400C Professional (Bosch) with an IR sensor of 160×120 pixels, of 0.1°C resolution, thermal sensitivity of <50 mK and measuring accuracy of IR ±3.0°. The emissivity value of 0.90 was used for gelatin.[16,17] The camera was fixed at a 15 cm distance above the sample and focused on the surface. The temperature effect of our APPJ in aqueous solution and liquid gelatin samples is summarized in the Supporting Information SI2 and has been reported earlier.[18]

Discoloration of inks in liquid samples was assessed using UV–Vis absorption spectroscopy. 200 μL of the sample to be analyzed were transferred to a UV-transparent 96-well plate (UV-star microplate; Greiner Bio-One GmbH) and the absorption spectrum was recorded between 250 and 800 nm with 1 nm resolution using a Synergy HTX Hybrid Multi Mode Microplate Reader (BioTek Instruments). Calibration lines were obtained using standard solutions of tattoo inks. The UV–Vis spectra of the 100 ppmv tattoo inks in phosphate buffer, are reported in Figure 1. The wavelength corresponding to the maximum pigment absorbance was selected for each ink for further analysis. For the black ink (Tribal) due to the absence of maxima in the visible range, we selected a wavelength close to the IR region (733 nm) to minimize interferences from other components of the ink. After plasma treatment, the absorbance spectra were carefully analyzed to check that no new band appeared in the ranges used for analysis.

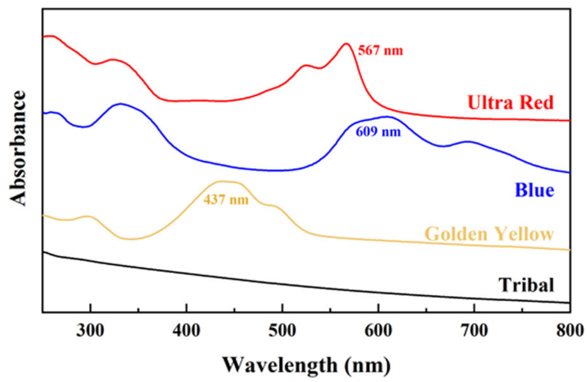


FIGURE 1 UV–vis spectra of untreated inks suspensions in phosphate buffer

The effect of NTP treatment on inks dissolved in solid gelatin disks was evaluated by reflectometry using a portable spectrophotometer (DataColor Check Pro Version 4.2.7).

3 | RESULTS AND DISCUSSION

APPJ was first tested for the discoloration of diluted tattoo inks (100 ppmv) in PB (Figure 2, left column). The suspensions were homogeneous immediately after preparation (Figure 2b), but some hours after preparation the pigment particles settled at the bottom of the container. For this reason, the suspensions were homogenized before each treatment and/or analysis.

The 100 ppmv ink suspensions in PB were subjected to APPJ treatment for selected times, up to 600 s (10 min). Immediately after the treatment, highly pigmented spots were evident at the interface and the remaining bulk solution was homogeneous for the four colors tested. The NTP treatment likely favors the aggregation of the water-insoluble pigments present in the inks, as already reported in the literature.[19] The samples were homogenized and analyzed by UV–Vis spectroscopy to obtain the relative amount of residual pigment in the solution (Figure 2c). In all cases, the pigment

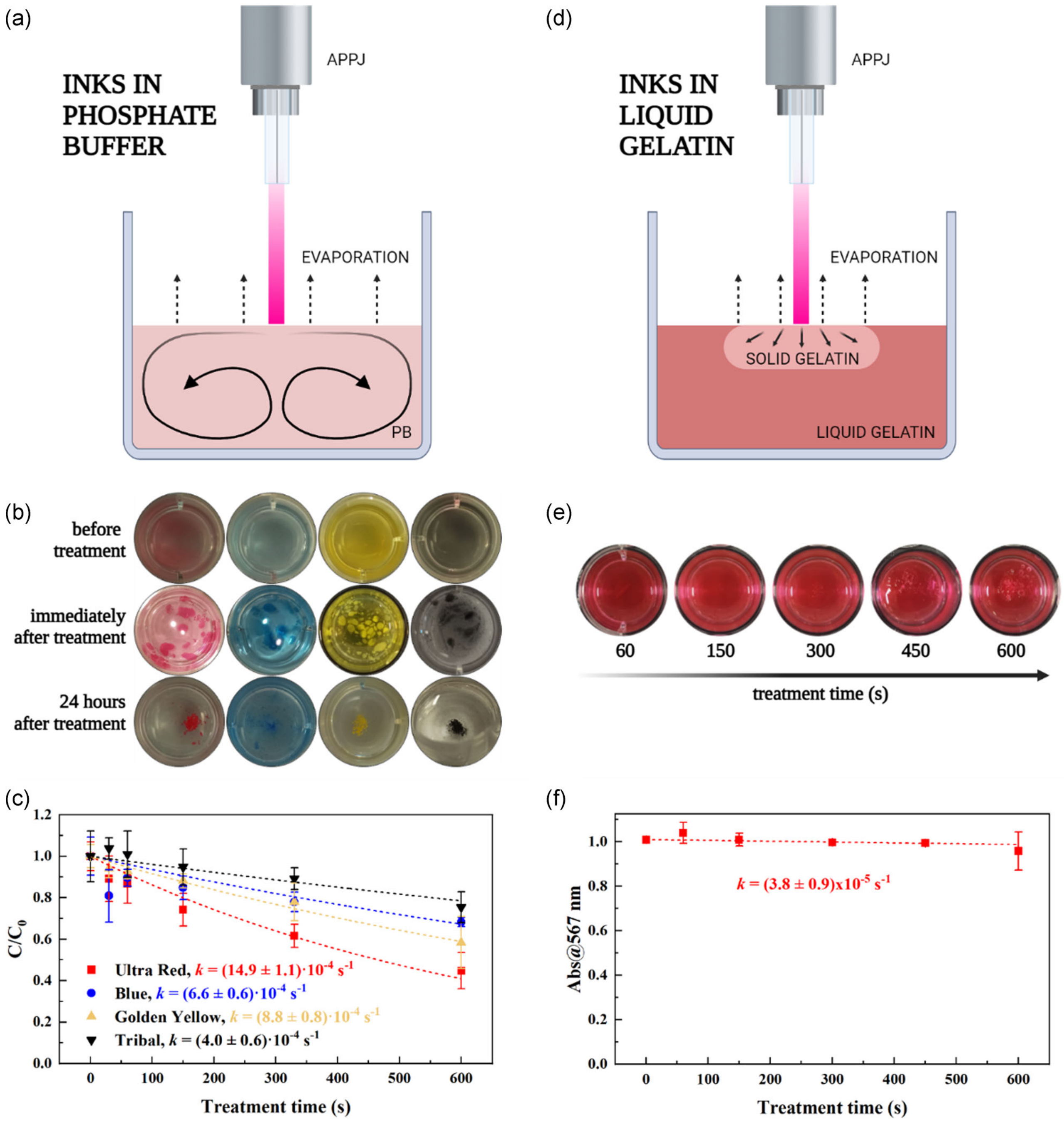


FIGURE 2 Effects of nonthermal plasma (NTP) treatment on 100 ppmv ink suspensions in phosphate buffer (PB) for red, blue, yellow, and black colors (left column) and on 100 ppmv red ink suspension in liquid gelatin (right column). (a) Scheme of the diffusion of RONS within the aqueous solution. (b) Pictures of ink suspensions in PB untreated, immediately after 600 s treatment and 24 h after treatment. (c) Normalized pigment residual concentration as a function of the treatment time. (d) Scheme of the diffusion of RONS within liquid gelatin solution. (e) Pictures of treated ink suspensions in liquid gelatin 0.02 g/mL. (f) The absorbance of treated ink suspensions at 567 nm as a function of the treatment time. The dashed lines are the best fit for experimental data obtained using exponential functions. Figure created with BioRender

concentration decreased with the treatment time. Kinetic constants for the pigment degradation were obtained by interpolating the experimental data using exponential decay functions and are indicated in the figure. The values correspond to half-life times between 7.8 min (red, fastest decomposition rate) and 30 min (black, slowest decomposition rate).

After showing that NTP can discolor tattoo inks water suspensions, we selected a model closer to a real tattoo application. One of the main components of the dermis (the central skin layer where the tattoo particles are placed) extracellular matrix is collagen (>70%).[20] We thus selected gelatin as a potential skin model for our study since it is derived from the partial hydrolysis of collagen. The right column of Figure 2 reports the results obtained by treating with APPJ 100 ppmv suspensions of Ultra Red ink in a diluted 0.02 g/mL gelatin solution. We show only the results obtained for the red ink since it is the one that is most affected by NTP treatment. The treatment does not significantly affect the color of the suspension in gelatin, even after 10 min (Figure 2e). As the NTP gas flow in contact with the surface of the liquid leads to evaporation, this produces a cooling effect[18] that is related to the gelling of the solution's surface (Figure 2d). This is related to the trapping of some air bubbles in the gelatin film produced, visible in the samples treated for longer times (Figure 2e). To ensure proper measurement, after the treatment the samples were warmed at 37°C, homogenized and analyzed by visible absorption spectroscopy. The quantitative data confirm that the effect of the NTP treatment on the color of the solution is negligible (Figure 2f). We estimate the half-life time for 100 ppmv red pigment treated with these conditions to be around 5 h. Thus, great differences are observed in the presence of components of the extracellular matrix of skin (gelatin), possibly related to the scavenging of short-lived reactive species by the biopolymer.

Finally, we exposed solid gelatin disks containing red tattoo ink to NTP treatment. In a solid sample, the plasma jet is expected to hit the interface region without any convection facilitating the diffusion of species in the bulk and concentrating all the energy delivered by NTP in a very small region (Figure 2, top right). This might cause a local temperature increase in this area. Gelatin disks (0.15 g/mL) containing 1000 ppmv Ultra Red ink, untreated, treated with area mode for 600 s and treated with point mode for different times (150, 300, 600, and 900 s) are shown in Figure 3a–c. Figure 3c shows that plasma treatment in point mode caused local melting of the gelatin disk and a partial burning in the contact point of the plasma plume, this is particularly evident for long treatments (enlargement in Figure 3d, see also Supporting Information SI3 and Figure S2). This was due to a very high local temperature increase of the gelatin film, up to 180°C. This temperature is responsible for the effect observed during point mode treatment and could possibly damage the skin in a real treatment application. To reduce this effect, we moved to area mode, to avoid the plume hitting the same spot of the target. With the area treatment mode, we intend to mimic what would be a real tattoo removal treatment. In the area treatment mode (Figure 3b) we could observe some local gelatin melting, but no burning effects. Temperature measurements confirmed that the temperature reached during the treatment, in this case, is lower (around 50°C, enough for melting the sample but not for burning it) and for very short fractions of seconds for each target point.

We measured the reflectance of the disks treated by area mode for 600 s with a colorimeter and we compared it to the untreated disks (Figure 3e). The curves in the two cases are superimposed and the only difference that was observed is due to the melting effect in the treated region (the treated disk is slightly brighter than the untreated one). Thus, the plasma treatment evaluated is not able to significantly degrade the tattoo ink pigment in the 0.15 g/mL gelatin disks in 10 min. This was confirmed

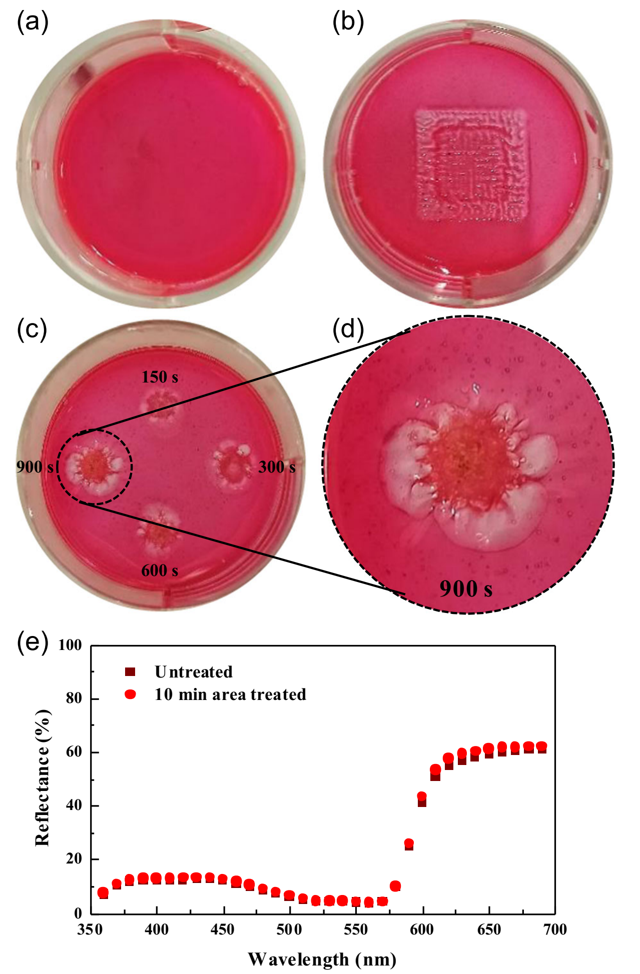


FIGURE 3 Effects of plasma treatment on 1000 ppmv Ultra Red ink suspension in 0.15 g/mL gelatin solid disks. (a) Untreated disk. (b) Disk treated for 600 s in area mode. (c) Disk treated for different times (150 s, 300 s and 600 s) in point mode. (d) enlargement of the 900 s treatment region in point mode. (e) comparison of the reflectance (350–700 nm) of the untreated disk and 600 s treated disk (area mode)

also for longer treatment times, up to 30 min (Supporting Information SI4).

Preliminary experiments on solid gelatin disks, obtained with another plasma jet (kINPen) working with argon as feed gas in point mode and using different nozzle-to-target distances (10 and 7 mm), showed similar results (see Supporting Information SI5 and Figure S4).

It is important to recall that all these experiments have been performed with very diluted ink suspensions (10^3–10^4 times) while tattoo inks are not diluted when they are applied to the skin. However, a study estimated that the maximum decrease in pigment concentration in the skin is 87%–99% many years after tattooing.[21] This means that the pigments in our model experiment were 10–1000 times less concentrated than in a real application case. Several works focused on advanced oxidation processes for dyes and pollutant decomposition reported a linear dependence of the degradation constant with the inverse of the starting concentration.[22,23] By assuming the same behavior, on the basis of the half-life time that we measured in the experiment reported in Figure 2d for liquid gelatin samples (about 5 h), we can conclude that the discoloration of tattoo pigments by reaction of the oxidant species generated by plasma would be practically impossible (a treatment should last 20–200 days). To increase the NTP oxidative effect more energetic treatment would be required (higher applied power or treatment time), but this could also lead to the potentially harmful thermal effects observed in the solid gelatin disks (Figures 3 and S3).

4 | CONCLUSION

Our research regarding tattoo removal from the skin with NTP jet treatment revealed serious limitations because: (i) to achieve effective degradation, specific wavelengths are needed; and (ii) the extracellular matrix of the skin, modeled here with gelatin films, significantly scavenges the effects of NTP (both with liquid and solid gelatin). Thus, NTP cannot be considered a standalone option for tattoo removal.

ACKNOWLEDGMENTS

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (Grant agreement No. 714793). The authors acknowledge MINECO for PID2019-103892RB-I00/AEI/10.13039/501100011033 project. The authors belong to the SGR2017-1165. Support for the research of Cristina Canal was received through the ICREA Academia Award for excellence in research, funded by the Generalitat de Catalunya.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

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How to cite this article: F. Tampieri, A. G. Araguz, C. Canal. Can we remove tattoos with non-thermal atmospheric plasma?. *Plasma Processes Polym*. **2022**;19:e2100188. https://doi.org/10.1002/ppap.202100188