Acellular Model For Evaluating Laser-induced Photothermolysis Of Tattoo Pigments

Elisa Moretti · Dario Allevi · Valerio Pedrelli · Maurizio Stefano Galimberti · Vincenzina Barbera

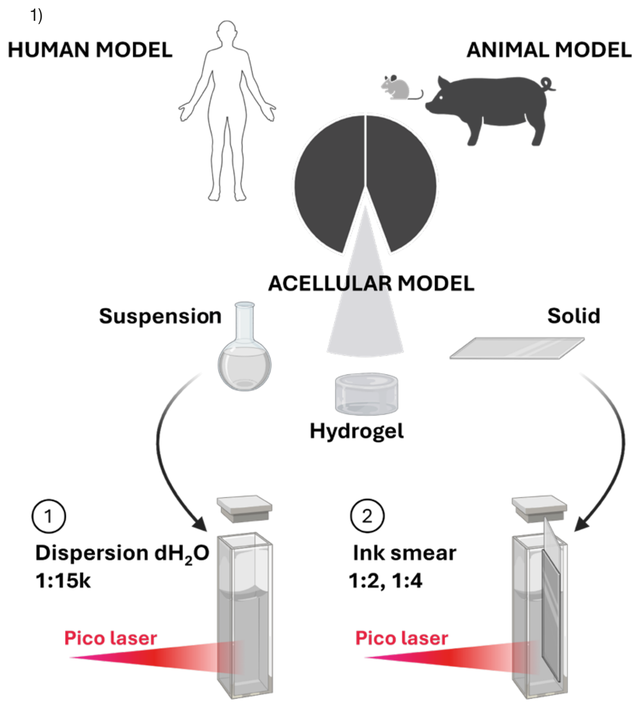
ABSTRACT

Laser tattoo removal relies on selective photothermolysis, yet the physicochemical mechanisms underlying pigment degradation remain poorly understood due to the lack of standardized in vitro models. This study presents **an acellular platform to investigate laser–pigment interactions using carbon black (CB) and TiO_2-based tattoo inks. Two experimental setups were employed: diluted inks in aqueous suspension and solid-state inks fixed on glass supports, both irradiated with picosecond laser pulses.** Dynamic light scattering (DLS), High-resolution transmission electron microscopy (HR-TEM), X-Ray Diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) analyses revealed that while TiO_2 remained unaltered, CB underwent significant morphological changes - such as graphitization and the formation of lamellar and cave-like structures - without clear evidence of fragmentation or aggregate size reduction, contrary to common assumptions. These findings demonstrate the need of acellular models in capturing pigment-specific responses to laser exposure and provide a foundation for future studies.

INTRODUCTION

Laser tattoo removal is based on selective photothermolysis, wherein targeted laser wavelengths induce photothermal and photoacoustic fragmentation of pigment particles. While clinical and ex vivo studies have elucidated physiological effects, the absence of standardized acellular models limits detailed mechanistic investigations at the physicochemical level. Recent advances have introduced thermochromic phantoms capable of mapping thermal distributions during laser irradiation; however, their application has been largely confined to laser ablation models. This study aims to develop and validate acellular systems (Fig. 1) for quantifying tattoo pigment photothermolysis, focusing on fragmentation efficiency and physicochemical characterization of degradation products. Two experimental models were employed: (1) tattoo inks diluted in water and (2) solid-state inks fixed on glass holders. Both were irradiated within quartz cuvettes to avoid interference from container absorption. Post-treatment, carbon black-based inks (responsive to laser) and TiO_2-based inks (resistant, negative control) were analyzed to assess pigment clusters size and morphological alterations.

1)



METHODS

1) PIGMENT EXTRACTION

- Dry black and white tattoo inks
- Double-wash solids with acetone and dichloromethane

2) PIGMENT CHARACTERIZATION

- ***Morphology and Size:*** HR-TEM, DLS (aggregate size, BSU size)
- ***Surface Charge:*** Zeta potential (DLS)
- ***Crystallinity:*** ImageJ analysis (HR-TEM) + XRD
- ***Surface Chemistry:*** FTIR (KBr pellet method)

3) LASER ACELLULAR MODELS

Set up 1: Ink dispersion in water

- Dilute tattoo ink 1:15,000 in deionized water
- Place 1 mL dispersion in quartz cuvette
- Laser treatment: 10 picosecond pulses at different fluences

Setup 2: Ink on Glass Holder

- Dilute ink (1:2 for black, 1:4 for white)
- Smear 1.5 µL on 0.9 × 5 cm glass holder
- Thermal fixation with an air gun (1 min)
- Insert the holder into quartz cuvette filled with 1 mL deionized water
- Laser treatment: 10 picosecond pulses at different fluences

4) PHOTOTHERMOLYSIS PRODUCTS CHARACTERIZATION

- Collect treated water
- Analyze aggregate size (DLS)
- Analyze morphology (HR-TEM)

RESULTS

CARBON BLACK CHARACTERIZATION

Black ink-derived carbon black aggregates were first characterized. The low-magnification **TEM** image (Fig. 2) shows nearly **spherical nanoparticles** with a basic structural unit (BSU) size of ~30 nm. DLS analysis (Tab. 1) indicated an average aggregate size of ~130 nm with a **good colloidal stability.**

2)

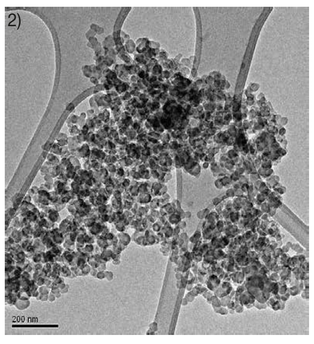
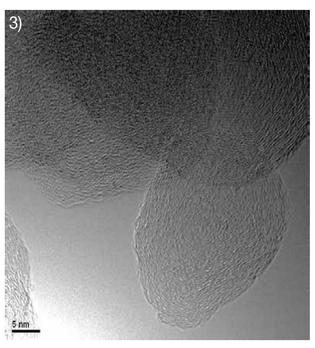


Table 1)

Size (d.nm)	St.dev (d.nm)	PdI
128,1	52,68	0,153
Z. Potential (mV)	St. dev (mV)	Cond. (mS/cm)
-17.9	7,73	0,0135

The **crystalline structure** of CB was analyzed by high-resolution TEM and XRD. The **TEM** image (Fig. 3) **reveals curved, concentric graphitic layers, typical of a turbostratic carbon structure**, with partial but not long-range order. **XRD** analysis (Fig. 4, Tab. 2) confirmed the **disordered structure** of CB pigment consisting of **small, defective graphitic domains embedded in an amorphous matrix.**

3)



4)

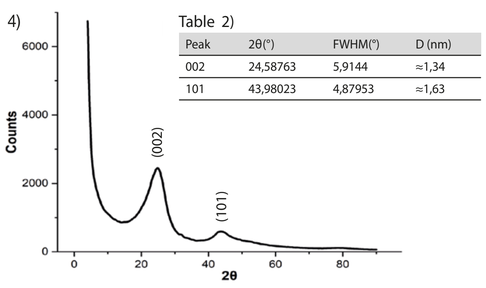
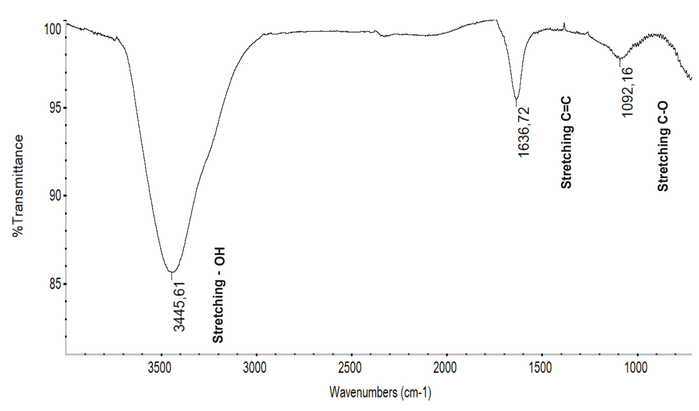


Table 2)

Peak	2θ(°)	FWHM(°)	D (nm)
002	24,58763	5,9144	≈1,34
101	43,98023	4,87953	≈1,63

Lastly, chemical characterization by **FTIR** (Fig. 5) revealed peaks at 3445 cm^{-1} (O–H stretching or adsorbed water), 1636 cm^{-1} (C=C stretching in graphitic domains), and 1092 cm^{-1} (C–O stretching), confirming the **presence of hydroxyl groups and oxygen-containing functionalities** on the carbon black surface.

5)



TiO_2 CHARACTERIZATION

TEM analysis (Fig. 6) shows that TiO_2 particles from tattoo ink form dense aggregates composed of **spherical, ellipsoidal, and faceted prismatic nanoparticles**, with primary sizes ranging from tens to a few hundred nanometers, typical of rutile morphology. **DLS** measurements (Tab. 3) revealed an average hydrodynamic diameter of ~ 370 nm and a **limited colloidal stability.**

6)

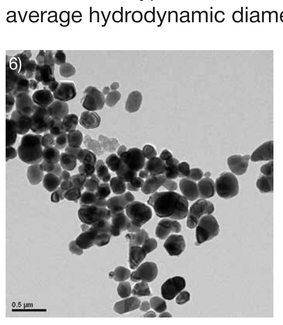
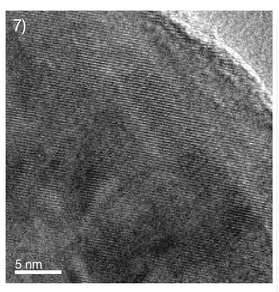


Table 3)

Size (d.nm)	St.dev (d.nm)	PdI
372,7	5,163	0,147
Z. Potential (mV)	St. dev (mV)	Cond. (mS/cm)
0.14	0,203	0,0003

The **crystalline structure** of TiO_2 particles was analyzed by high-resolution TEM and XRD. **TEM** images (Fig. 7) show **ordered lattice fringes**, depicting high crystallinity. XRD analysis (Fig. 8, Tab. 4) identifies rutile TiO_2, with sharp peaks. The (110) peak, shifted to 2θ ≈ 26.7°, suggests slight lattice distortion. Crystallite sizes range from ~1.6 to 2.3 nm, confirming **highly crystalline, slightly strained rutile domains.**

7)



8)

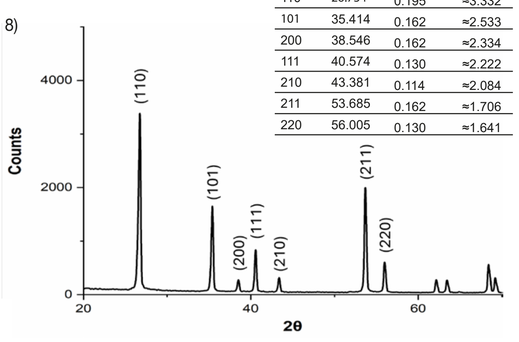
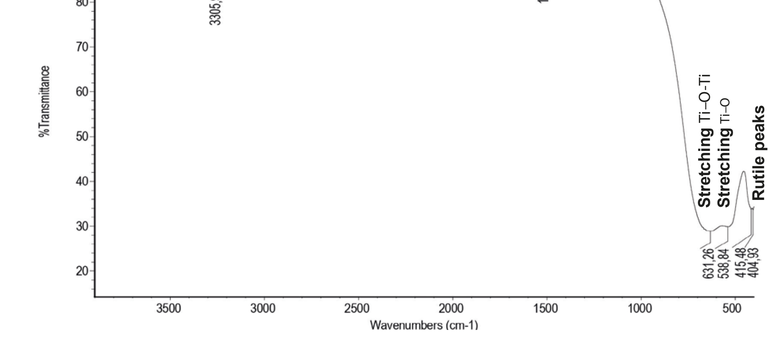


Table4)

Peak	2θ(°)	FWHM(°)	D (nm)
110	26.734	0.195	≈3.332
101	35.414	0.162	≈2.533
200	38.546	0.162	≈2.334
111	40.574	0.130	≈2.222
210	43.381	0.114	≈2.084
211	53.685	0.162	≈1.706
220	56.005	0.130	≈1.641

The **FTIR spectrum** (Fig. 9) shows peaks at 538.84 and 631.26 cm^{-1} (Ti–O and Ti–O–Ti stretching) and at 404.93 and 415.48 cm^{-1}, characteristic of rutile TiO_2 with low- and mid-frequency Ti–O vibrations, suggesting microstructural diversity. **The spectrum confirms the rutile structure**, with possible nanoscale variations due to crystallite size or lattice strain.

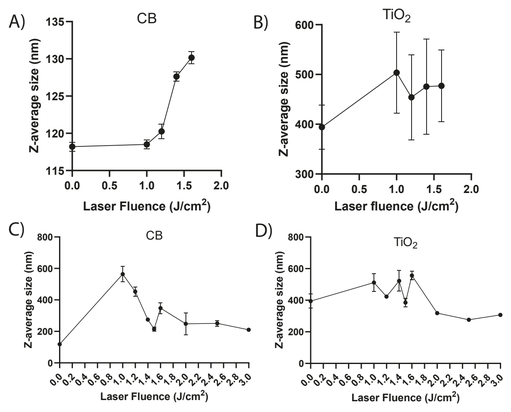
9)



PHOTOTHERMOLYSIS PRODUCTS CHARACTERIZATION

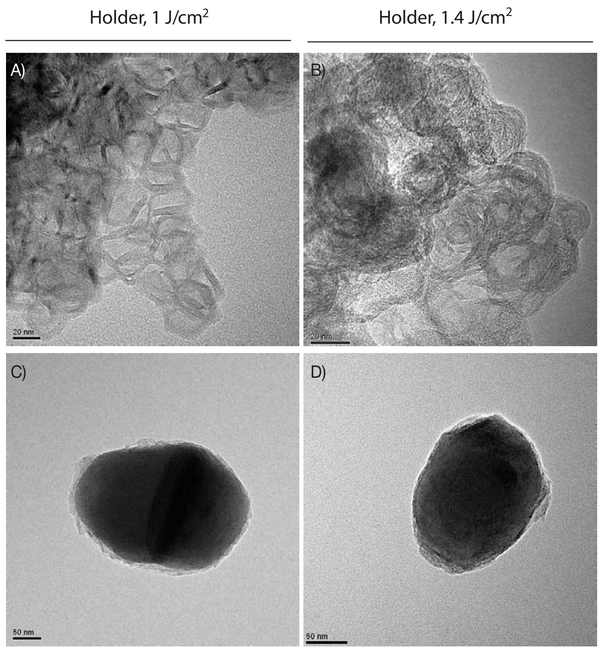
Firstly, **DLS** analysis evaluated the **Z-average size** of CB and TiO_2 **aggregates after pulsed laser treatment** in two setups. **CB in suspension** (Fig. 10 A) showed an **increase in cluster size** with rising laser fluence, suggesting laser-induced aggregation. **CB fixed** on a glass holder (Fig. 10 C) resulted in a **decreased cluster size with fluence**, although the original size was not fully restored. **TiO_2 exhibited minimal size variation** (Fig.10 B,D), confirming its expected resistance to laser fragmentation, consistent with its role as a negative control.

10)



TEM analysis highlights a marked **morphological evolution of carbon black upon laser treatment, with TiO_2** (Fig.11C,D) **showing no structural changes.** At 1.0 J/cm² (Fig. 11A), CB undergoes partial graphitization and **hollow (cave-like)** particles. Increasing the fluence to 1.4 J/cm² (Fig. 11B) results in larger lamellae arranged in **spiral, rose-like patterns**, likely due to laser-induced cavitation. At 3.0 J/cm², no CB aggregates were observed, possibly due to ablation or low residual concentration.

11)



CONCLUSIONS

This study confirms the feasibility of an acellular model to investigate laser-induced photothermolysis, enabling physicochemical analysis of pigment behavior. Contrary to literature expectations, **no fragmentation or size reduction of CB** aggregates was observed; instead, laser exposure induced **structural reorganization, including graphitization and the formation of lamellar and cave-like morphologies.** TiO_2-based inks remained unchanged, validating their role as a stable negative control. These findings highlight the value of controlled, acellular systems for mechanistic insight into laser-induced pigment transformations, beyond what clinical or ex vivo models can provide.

FUTURE PERSPECTIVES

Future perspectives include scaling up pigment quantities for laser treatment to enhance recovery and analysis of degradation products. Chemical comparisons between treated and untreated inks will be introduced, alongside crystallinity assessments. The model will also be extended to small molecule-based tattoo inks to study a broader range of photothermolysis behaviors. Finally, a thermochromic hydrogel model is being tested for temperature-mapping of the laser-pigments interaction.

YORE
Biotech Tattoo Removal

LASER PLAST

POLITECNICO MILANO 1863

We are looking for collarations. Contact us at: Info@yorebiotech.com