

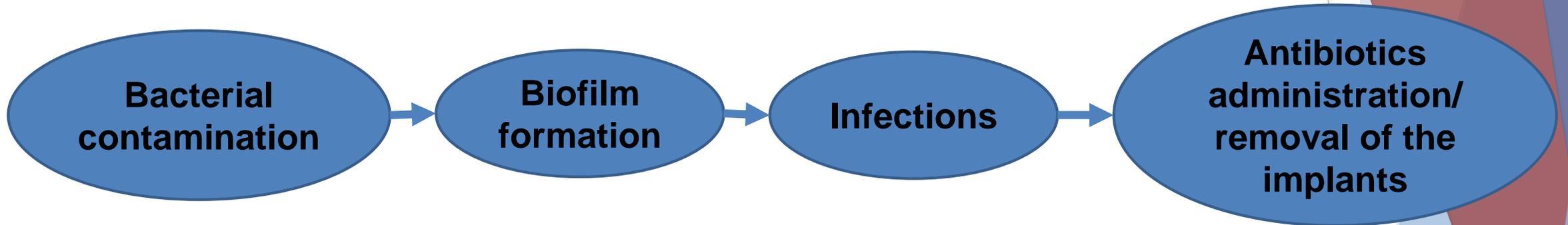


Surface coating and functionalization of metallic biomaterials with essential oils for antibacterial applications

Martina Cazzola¹, Sara Ferraris¹, Valeria Allizond², Giuliana Banche², Cinzia Margherita Berteza², Giovanna Gautier di Confiengo³, Chiara Novara¹, Andrea Cochis⁴, Lia Rimondini⁴,
Silvia Spriano¹

martina.cazzola@polito.it

ISSUE: bacterial infections in biomedical devices



- ▶ **Epidemiological impact** (morbidity and mortality)
- ▶ **Economic impact** (prolonged time of hospitalization, loss of working days, increased use of diagnostic procedures, removal of the implant)

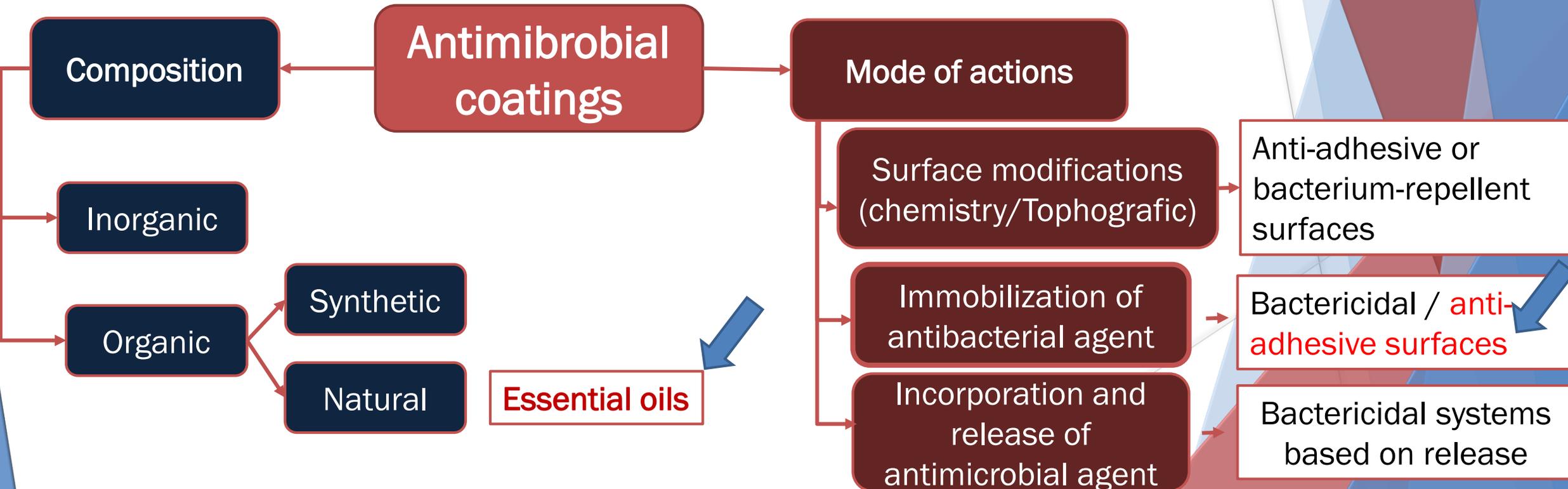
SOLUTIONS?

Antibiotic therapy



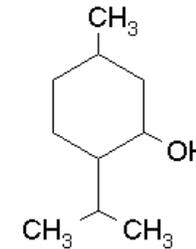
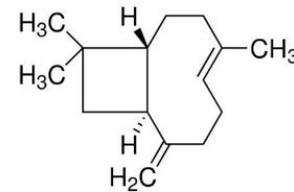
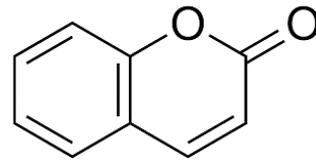
The systemic use leads to:

- Development of resistant bacterial strains
- Side effects



ESSENTIAL OILS

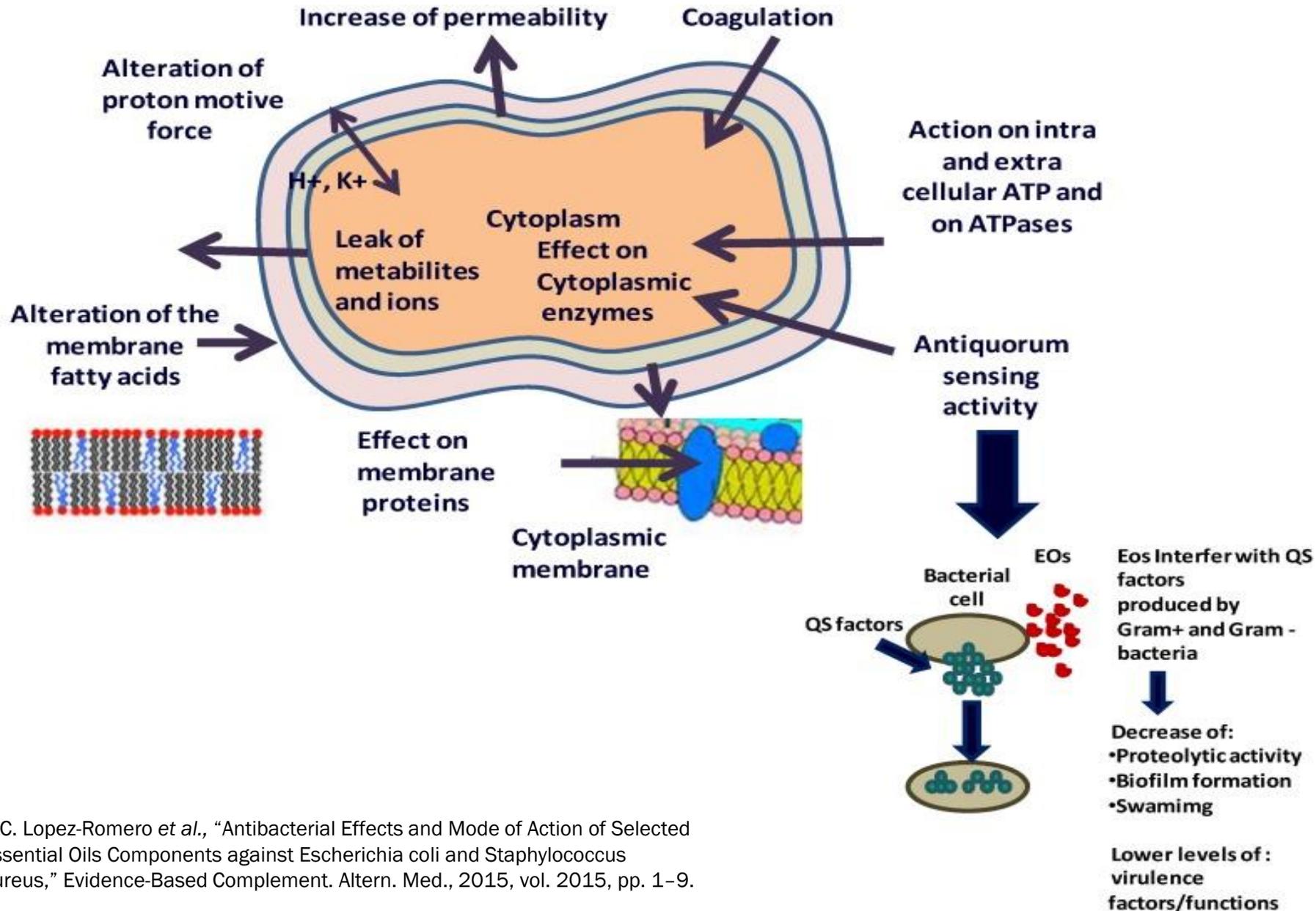
DEFINITION: product obtained from a **natural raw material** of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase by physical processes(ISO/D1S9235.2)



COMPOSITION

- TERPENS (monoterpens e sesquiterpens)
- TERPENOIDS (alcohols, aldehydes, chetons, esters, oxides)
- PHENYLPROPANOIDS (coumarins, fenilpropeni, hydroxycinnamic acids)
- SULPHUSES AND NITROGEN COMPOUNDS

MECHANISMS OF ACTION OF ESSENTIAL OILS



J. C. Lopez-Romero *et al.*, "Antibacterial Effects and Mode of Action of Selected Essential Oils Components against *Escherichia coli* and *Staphylococcus aureus*," Evidence-Based Complement. Altern. Med., 2015, vol. 2015, pp. 1-9.

Aim of the work

- The relevance of the topic of this work deals with the need for innovation in the **development of anti-adhesive surfaces**, since a range of medical, laboratory and general purpose instrumentation requires antibacterial safety.
- To this aim, stainless steel **316L** and **Ti6Al4V**, which are alloys used in the fabrications of different devices, were **coated or functionalized with *Mentha piperita* essential oils**.
- The procedure of coating was developed for non-implantable device, while functionalization of the surface of chemically treated Ti6Al4V (CT) was developed for implant applications in contact with bone.

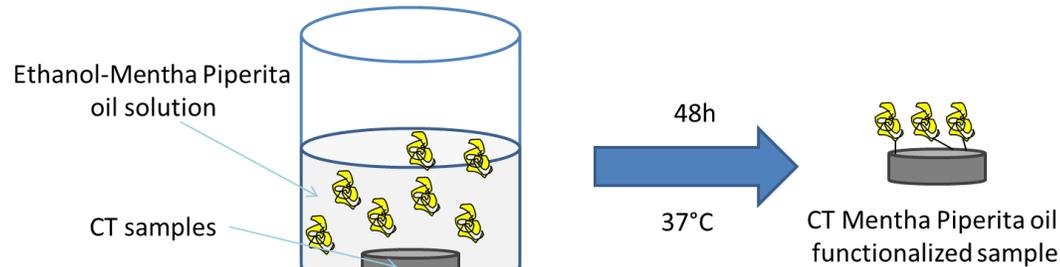
MATERIALS AND METHODS

SAMPLES:

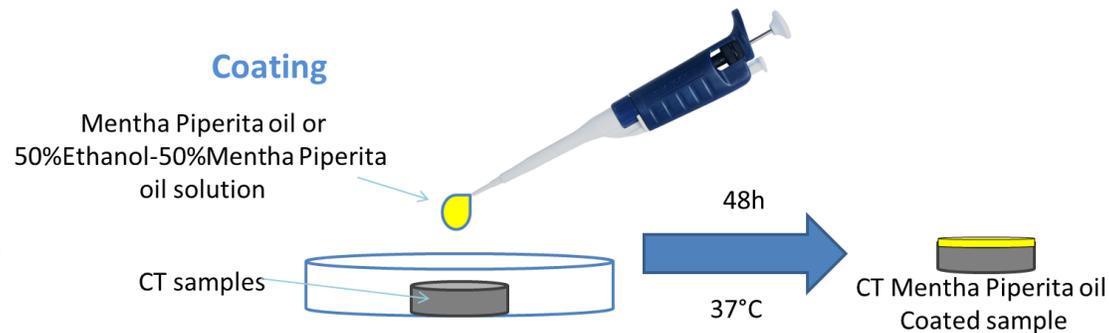
- Ti6Al4V ALLOY
- Ti6Al4V ALLOY CHEMICALLY-TREATED (CT)
- Stainless steel 316L

SYNTHESIS METHODS

Functionalization

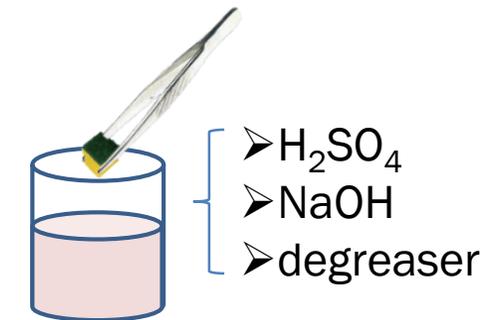


Coating

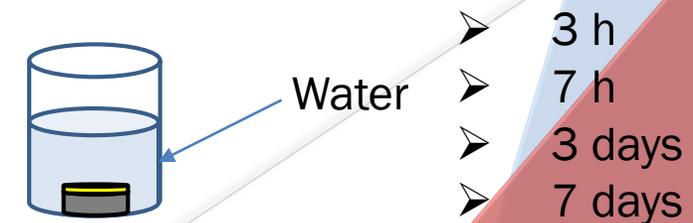


PROCEDURES FOR THE ANALYSIS

Washing

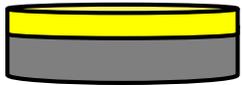


Release in water



CHARACTERIZATIONS OF THE COATINGS

Coating



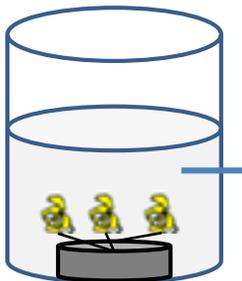
- Fluorescence microscopy (mint autofluorescence)
- XPS
- FTIR
- Tape test (coating adhesion)
- Antibacterial test

Coating after water washing

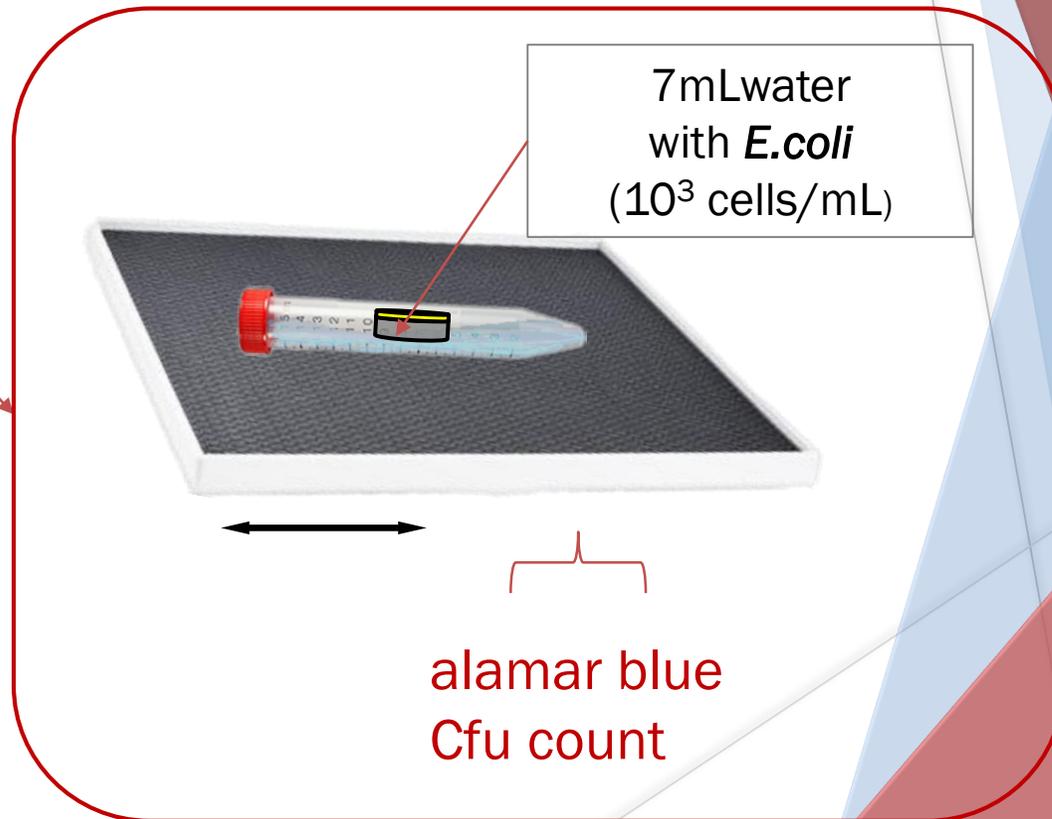


- FTIR
- Tape test

Functionalization



XPS

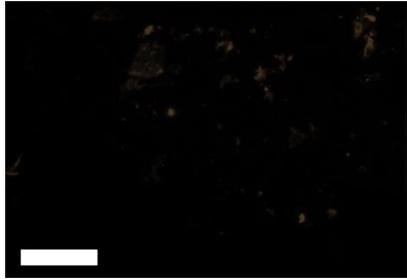


7mL water
with *E.coli*
(10³ cells/mL)

alamar blue
Cfu count

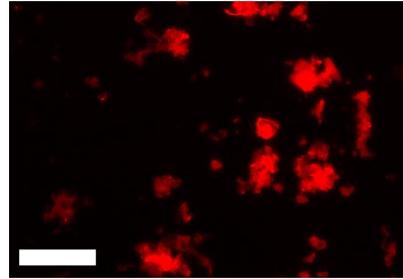
Fluorescence microscope observations

Ti



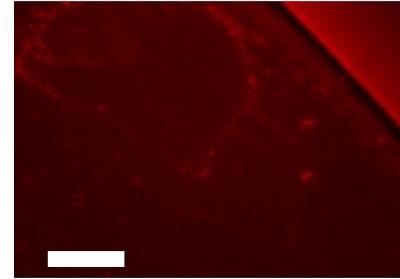
100μm

Ti_mint



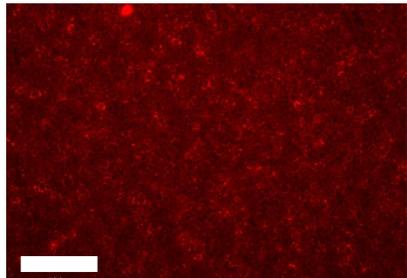
100μm

Ti_mint_rel3h



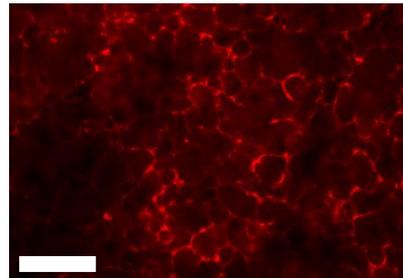
100μm

Ti_mint_rel7h



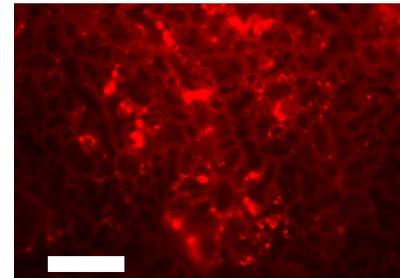
100μm

Ti_mint_rel3d



100μm

Ti_mint_rel7d



100μm

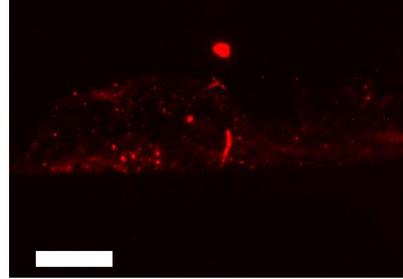
Fluorescence microscope observations

steel



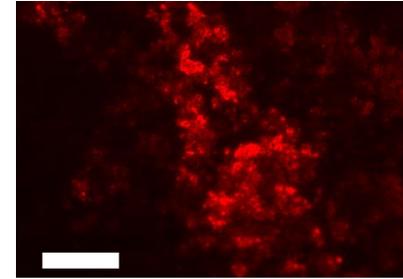
100μm

steel_mint



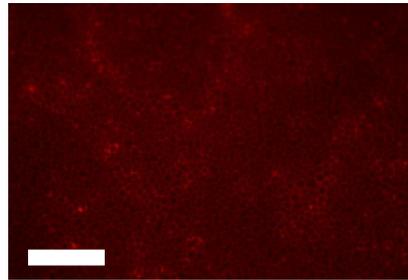
100μm

steel_mint_rel3h



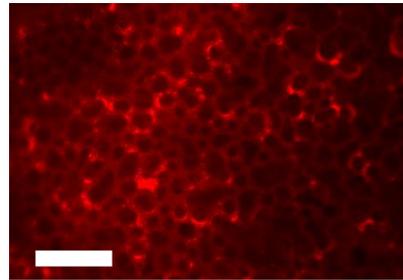
100μm

steel_mint_ril7h



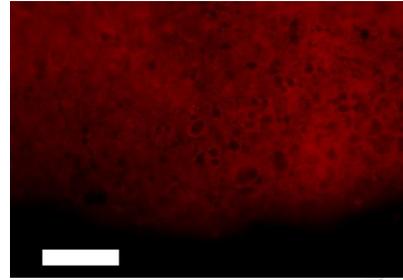
100μm

steel_mint_rel3d



100μm

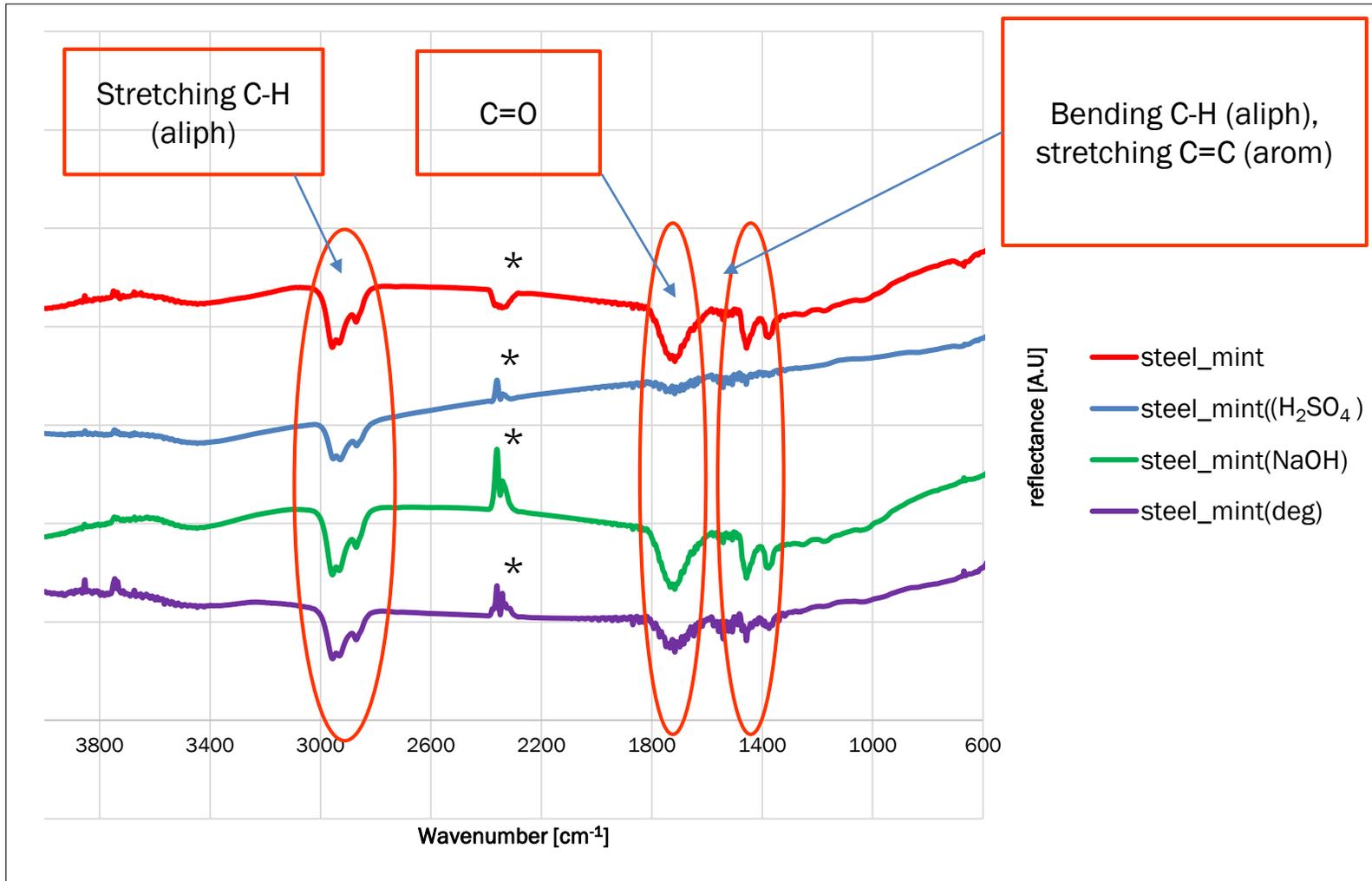
steel_mint_ril7d



100μm

A fluorescent layer is visible on both Ti alloy and steintess steel samples coated with mint oil, also after 7 days of release in water

FTIR measurements on steel samples

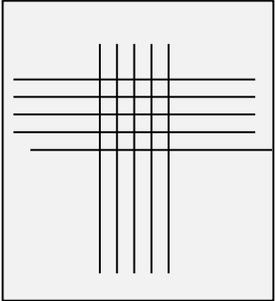


Peaks	Assignments
3450–3200 cm ⁻¹	stretching –OH
2960–2870 cm ⁻¹	stretching C–H
1780–1700 cm ⁻¹	stretching C=O
1450–1370 cm ⁻¹	bending C–H (aliph), stretching C=C (arom)

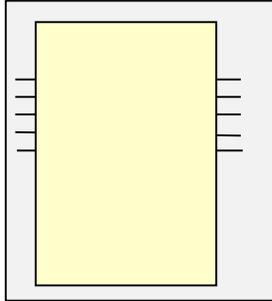
The spectra of all the samples are characterized by the typical vibrational peaks of oxygenated monoterpenes

Tape Test (ASTM D 3359)

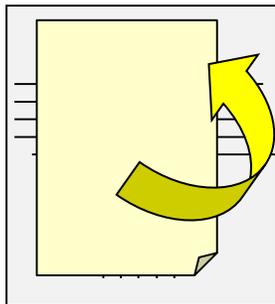
a. Cross-cut area preparation



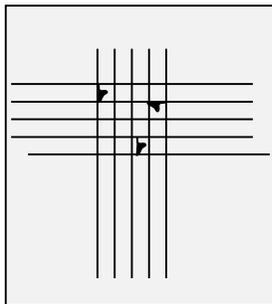
b. Tape positioning



c. Tape removal



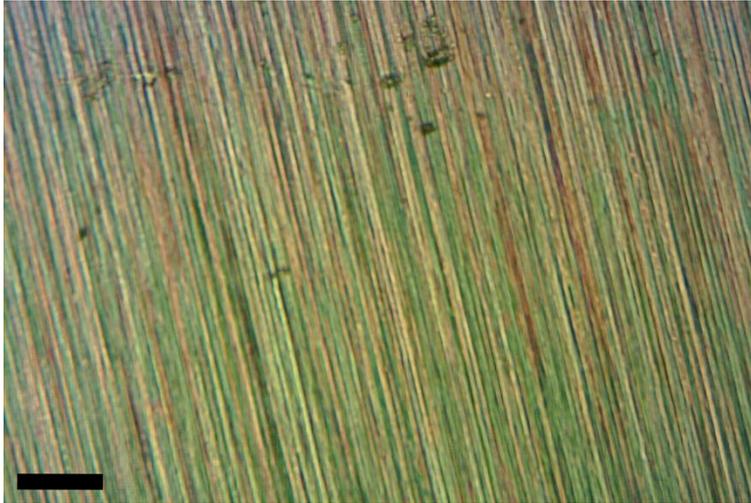
d. Observation



Classification	% of Area Removed	Surface of Cross-cut Area From Which Flaking has Occured for 6 Parrallel Cuts & Adhesion range by %
5B	0% None	
4B	Less than 5%	
3B	5 - 15%	
2B	15 - 35%	
1B	35 - 65%	
0B	Greater than 65%	

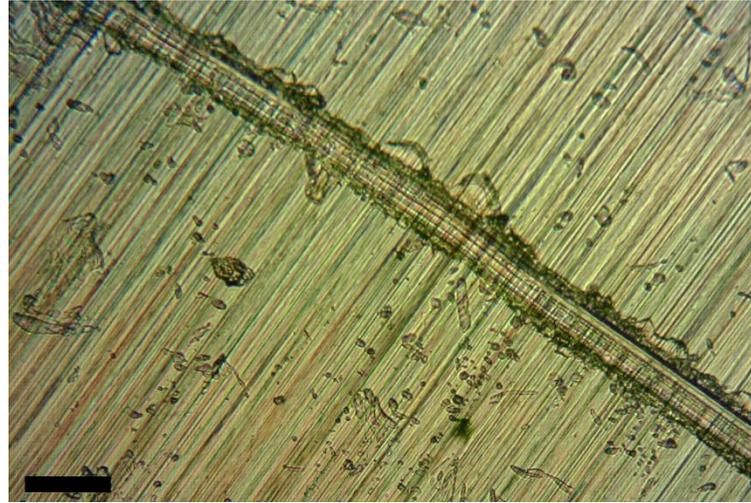
Tape Test (ASTM D 3359)

Before tape test



100μm

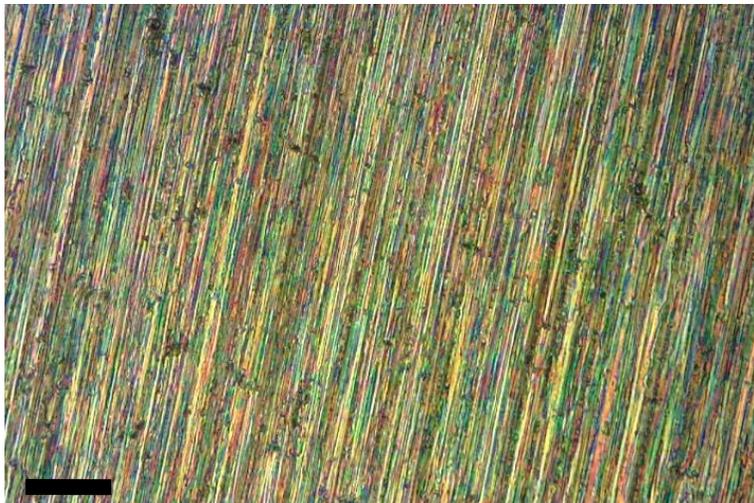
After tape test



100μm

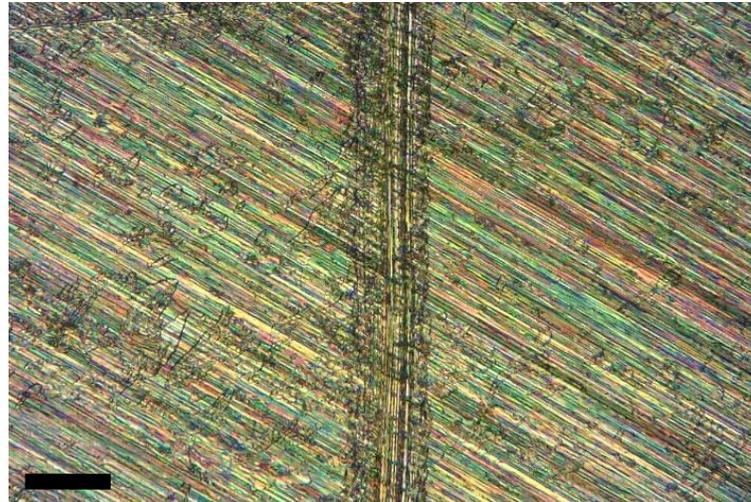
**Mint coating on
polished steel:
Classification 2B**

Before tape test



100μm

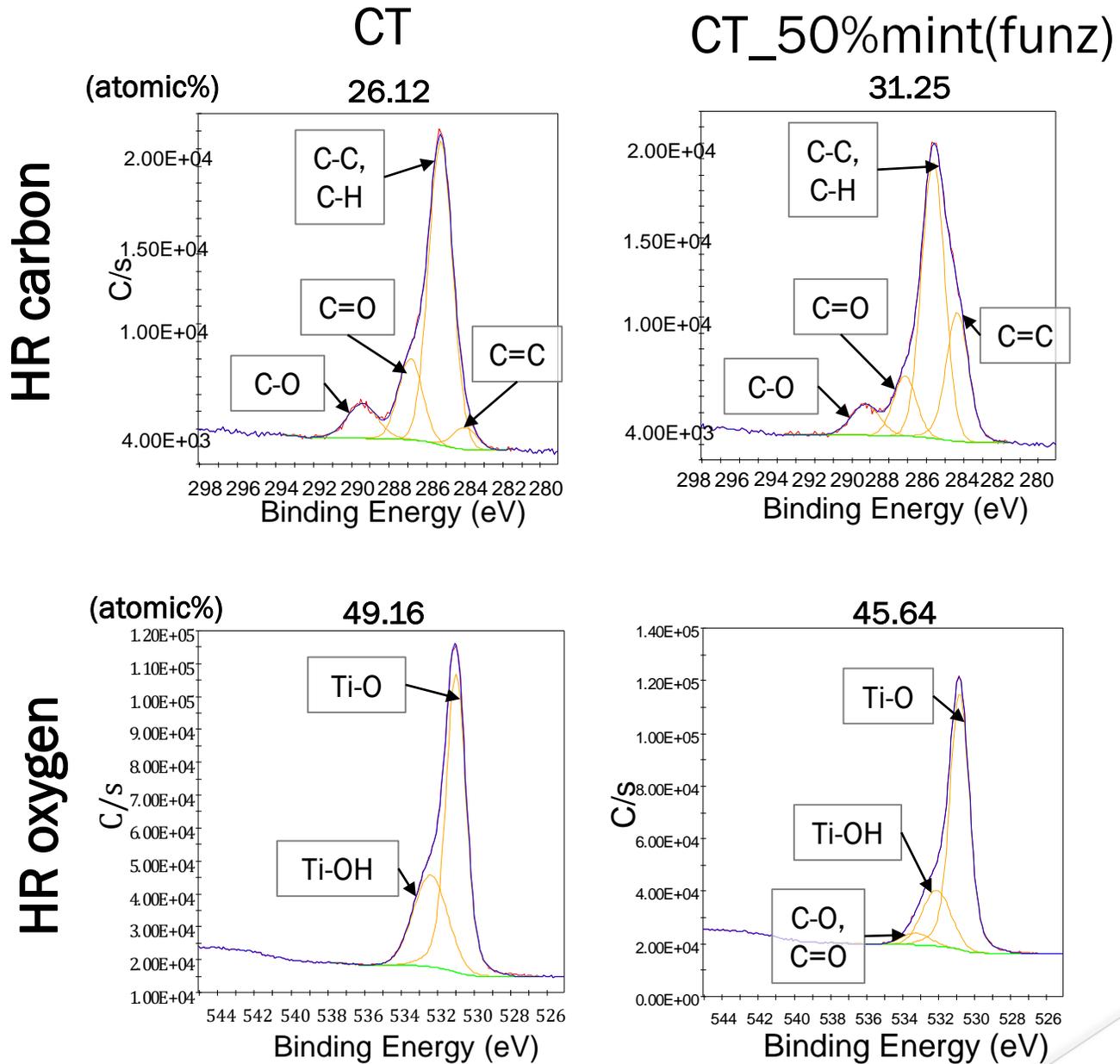
After tape test



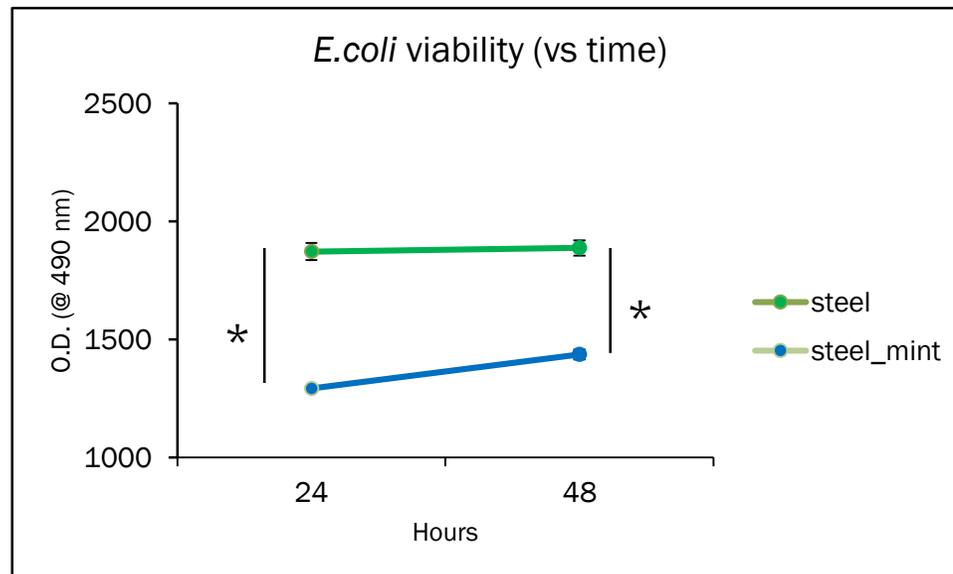
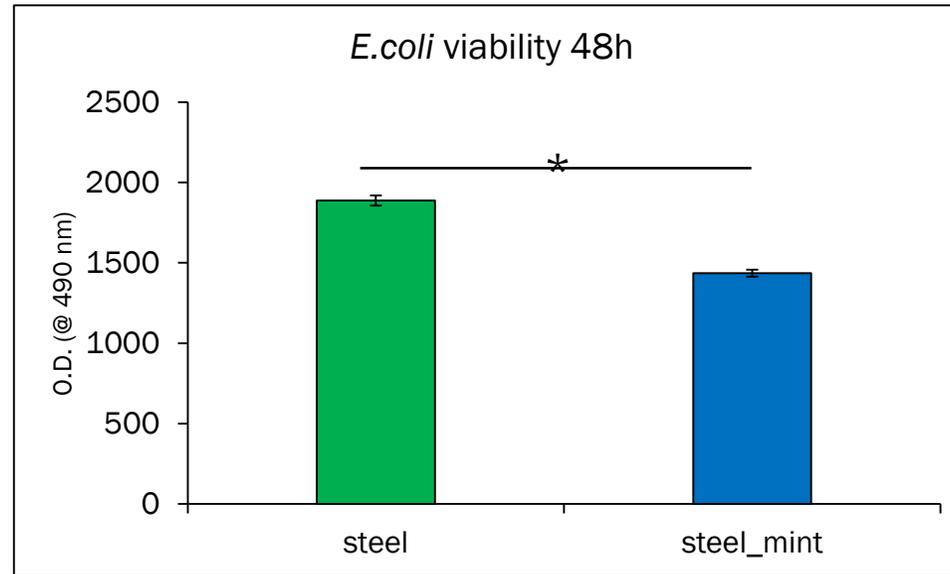
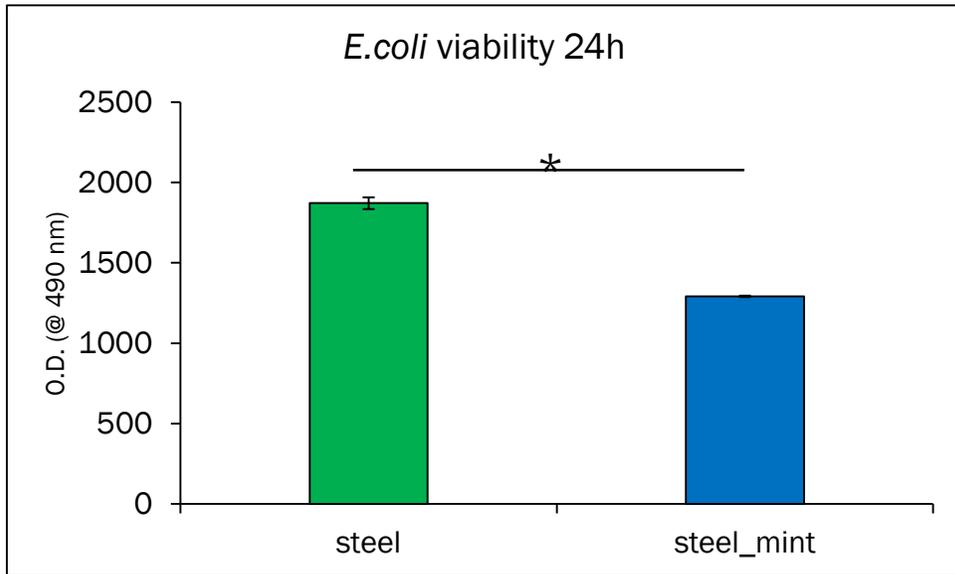
100μm

**Mint coating on high
roughness steel:
Classification 3B**

XPS on functionalized samples



Antibacterial test



The mint coating was able to reduce the amount of adherent metabolically active bacteria in a significant manner in comparison with bare control materials after 24 and 48 h of incubation

CONCLUSIONS

- ▶ The **success of functionalization** and **coating** treatments was highlighted by XPS or FTIR analysis that showed the presence of different biomolecules of the mint oil on the surface of the samples, according to the different procedures.
- ▶ The **coating** on both stainless steel and Ti6Al4V samples resulted **stable** also after 7 days of soaking in water, as observed by fluorescence microscopy. Moreover, the tape test performed on steel samples showed a good stability of the coating which was increased by a higher roughness of the surfaces before the coating procedure.
- ▶ The coated steel samples were also washed with acid and basic solutions in order to test the resistance to cleaning and analyzed by means of FTIR spectroscopy which showed that the **coating resists to basic cleaner** and has only a little damage after acid cleaning.
- ▶ The mint coating on steel showed the ability to **reduce the *E. coli* surface contamination** highlighting an anti-adhesive behavior.
- ▶ The procedure of coating and functionalization performed with an essential oil seems to be a **promising strategy to exploit their antibacterial activity** for both non-implantable and implantable applications in biomedical field and it can be easily extended to other essential oils.