

Spoke 4 – WP4

“Materials and micro-nanotechnologies for health applications”

-A summary of the 2nd year activities-

WP4 leader Silvia Scalese



WP4 speaker Nadia Lampiasi



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ECS00000022 PROJECT «SAMOTHRACE 2ND YEAR ACTIVITIES REVIEW»



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e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

- **WP4 leader: Silvia Scalese (IMM)**
- **10 CNR Partners:** IBF, IBSBC (ex IBFM), IC, ICB, IFT, IMM, IRIB, ISMN, ISP, URT-Labsens;
- **1 Affiliated:** STLab

Task 4.1 Nanomaterials-based Biosensing devices (IMM, IC, ISMN, IBF, IRIB, URT LABSENS, IFT)

Task 4.2 Development of devices for cancer diagnostics (STLab, IMM, IFT, IRIB, IBF, ICB)

Task 4.3 Development of portable medical imaging and smart healthcare systems and devices (IMM, STLab, IBSBC, IRIB, IFT)

Task 4.4 Environment-related health devices (IMM, IFT, ISMN, IC, ICB, IRIB, IRIB-ISP, IBF)

Task 4.1

Nanomaterials-based Biosensing devices

Application fields:

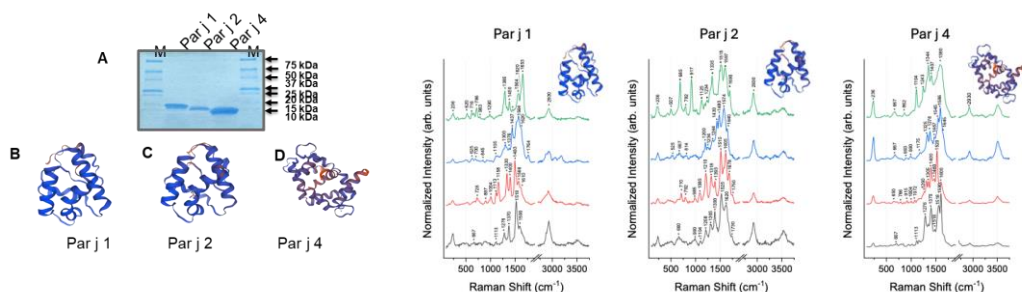
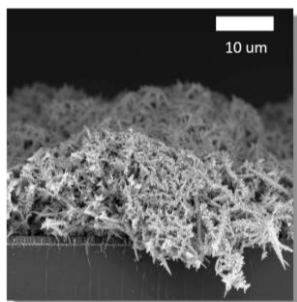
- *Parietaria Judaica* (Par j 2) pollen sensing

Sensitive spectroscopic detection based on plasmonic nanostructures /sensing of allergenic proteins

Clinical Need methods to accurately identify and characterize allergenic proteins present in pollens of plants, such as *Parietaria Judaica*. **Impact:** Advancements in accurate diagnosis and treatment of allergic rhinitis and asthma, alongside refined treatment approaches, will ultimately enhance patient outcomes and improve quality of life.

Development Status and Roadmap

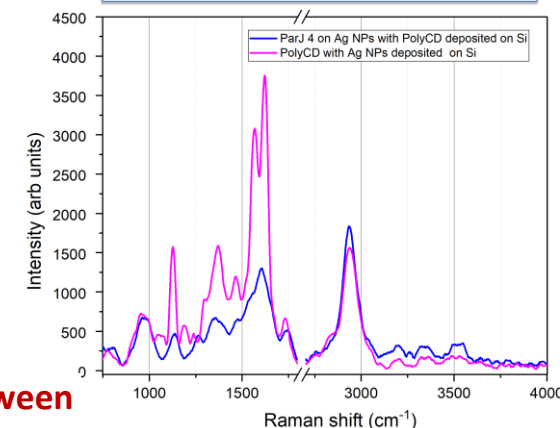
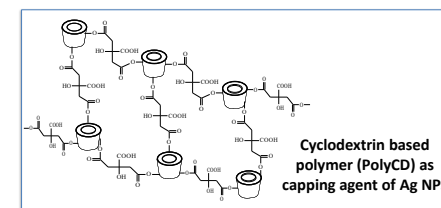
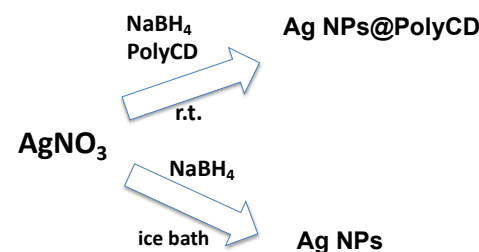
Raman fingerprint of three variants of *Parietaria j* protein by SERS from Ag dendrites



Paper submitted

Samothrace - ECS_00000022

SERS of *Parietaria j* protein from **Ag nanoparticles** functionalized by PolyCD (URT LabSens + IRIB PA + URT ISMN ME) .



Study of the interaction between PolyCD and recombinant *Par j* protein

Paper in preparation

On going TRL 3

Next step: “selectivity implementation” by suitable functionalization of plasmonic nanostructures.

Task 4.2

Development of devices for cancer diagnostics

Subtask 4.2.1 - Development of electrokinetic microfluidic devices for cells characterizations (STLab, UniCT (DIEEI, BIOMETECH) + ext. collaboration IRIB-CNR

Subtask 4.2.2 - Hybrid nanocomposites with magnetic properties for early diagnostics of cancer (*ISMN Messina, IMM Lecce, IRIB Palermo, IBF Palermo*)

Subtask 4.2.3 - Nanostructured systems engineered for selective recognition of cancer cells for precision diagnosis (*ICB, IRIB, IFT*)

Subtask 4.2.1: Development of microfluidic systems for the label-free separation of circulating tumour cells in peripheral blood

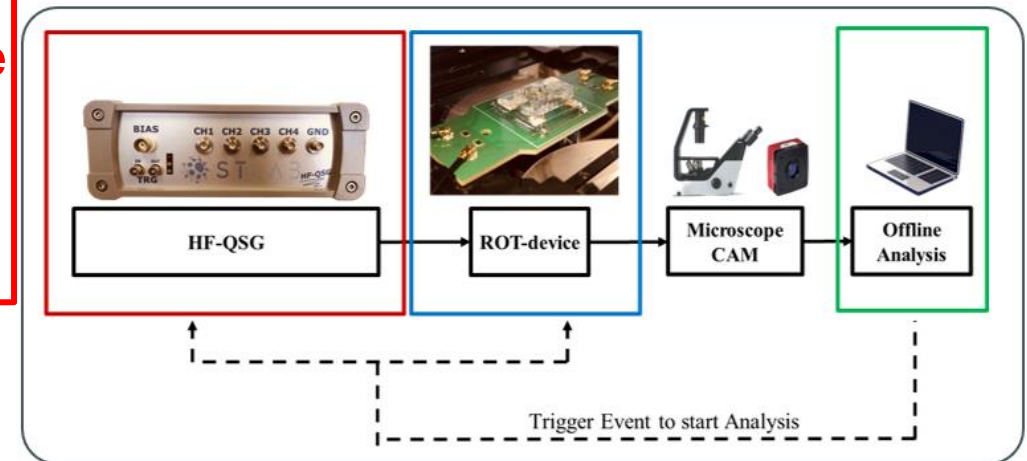
Development of cell-physiology platform based on electrokinetics methods for label-free characterization of cells

Collaboration network between Spoke 4 and Spoke 1. Activity details are shown in SPOKE 1 presentation

Overall goal: development of systems capable of quickly and effectively characterize and sort cells populations based on membrane' and cytoplasm' characteristics

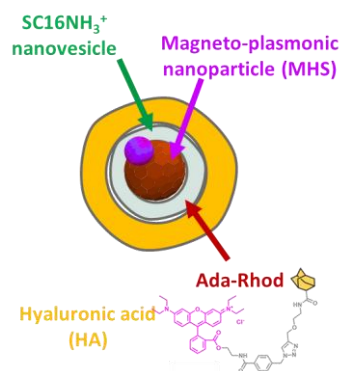
System description: the platform is composed by three highly customized elements developed within the project: (i) an electric generator, (ii) a BIO-chip, (iii) a video analysis algorithm

Technology transfer: the systems has been extensively tested in UniCT (BRIT). **It will be further tested within the SAMOTHRACE project at different CNR laboratories** to be then commercialized, initially as “for research use only”, to biological laboratories. The platform is expected to cost less (1/10) with respect to commercial alternatives which do not offer the same functionalities, in terms of throughput and automation.

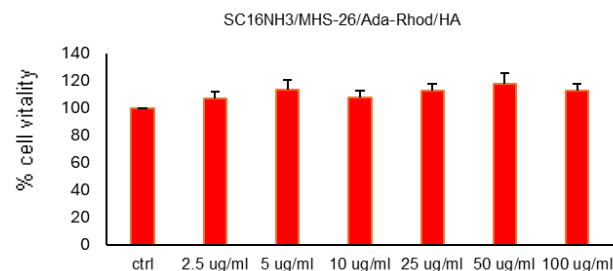
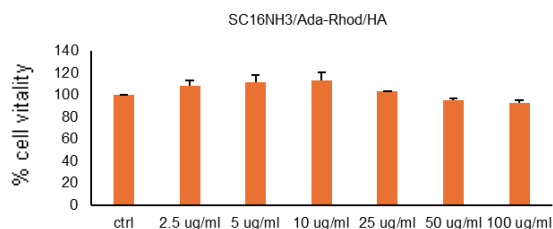


Clinical Need : Highly sensitive and accurate methods for the early melanoma diagnosis

Synthesis of SC16NH₃⁺ and nanohybridization process

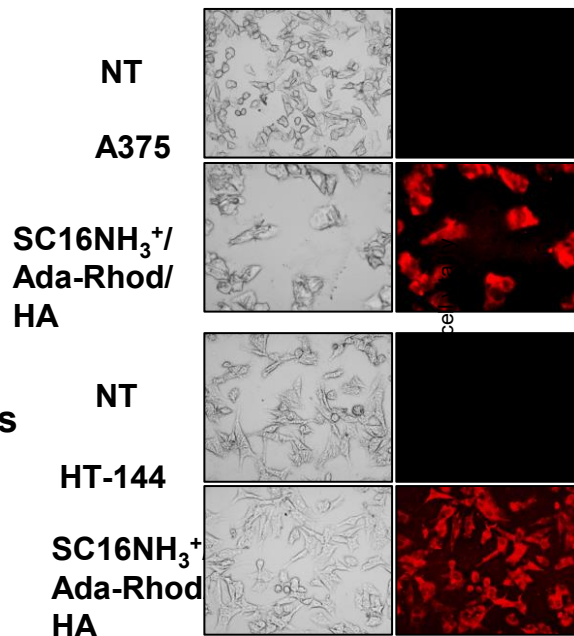


Citotoxicity assay vs. macrophages

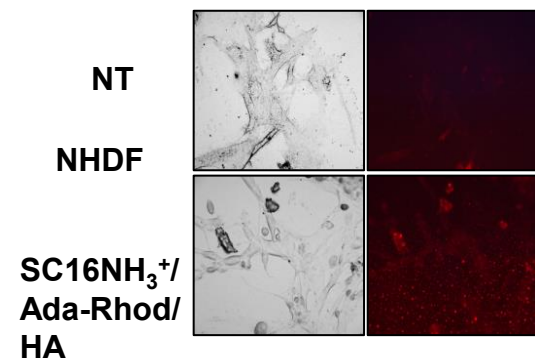


Cell interaction assays

Melanoma cells



Fibroblasts



Nanoparticles species enters cells and localizes in the cytoplasm of melanoma cells.

Specificity of the binding of the nanoparticles demonstrated by their inability to recognize the fibroblasts (control)

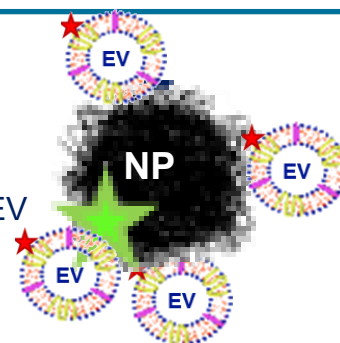
TRL:
Initial: 1;
ML12: 3;
End of project: 4-5

CHALLENGES

Validation of the platform on real samples.

Validation of the output in presence of interferences.

Interaction of amphiphilic cyclodextrin-based nanostructures (NP) with extracellular vesicles (EV)
NP = SC16NH₃⁺/Ada-Rhod – EV
= Algosomes w. Alexa647

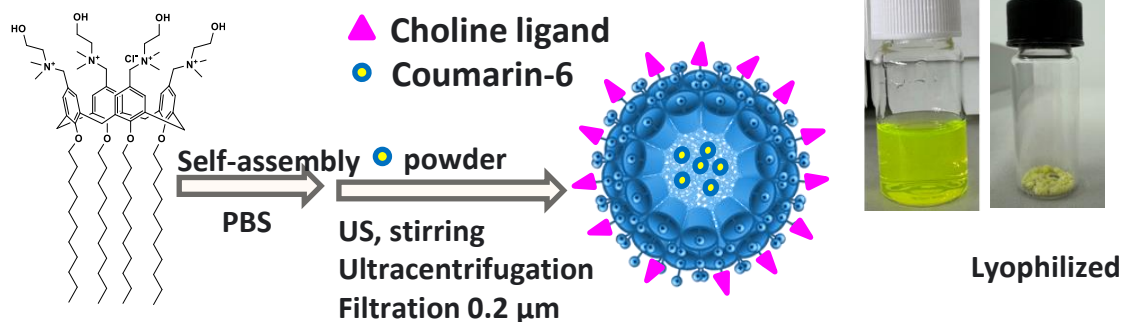


...next step

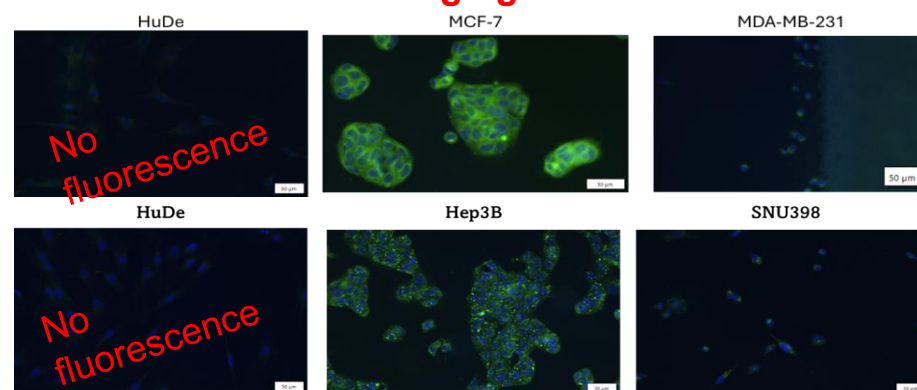
Investigation of interaction between functionalized nanoparticles and EVs from tumoral and non tumoral cell lines.

Clinical need: Need for more effective methods for accurate visualization of tumor cells relevant for diagnostic purposes and image-guided surgery.

Synthesis and characterization of a fluorescent and stable nanodevice exposing choline ligands

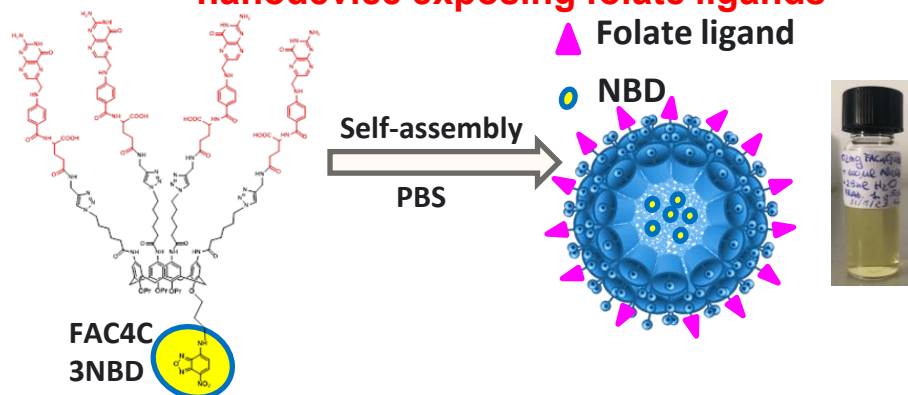


Selective fluorescence imaging of breast and HCC cells

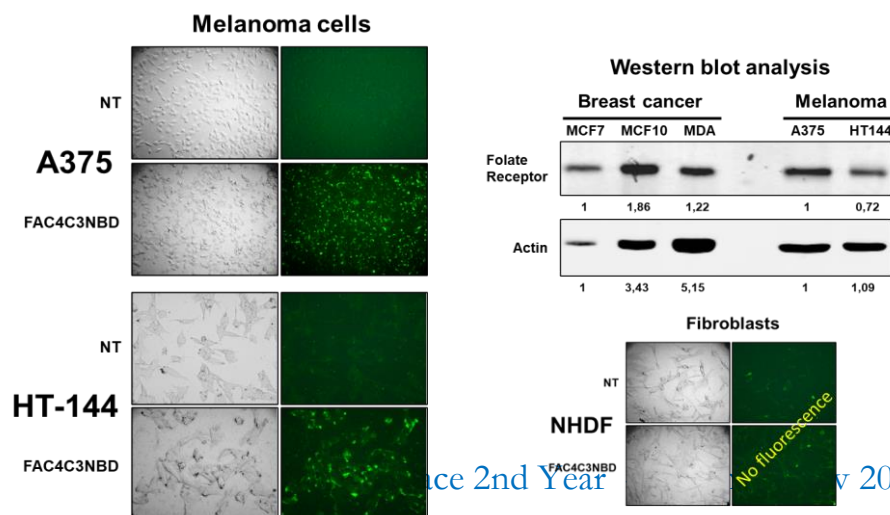


The fluorescent intensity inside the cells depends on the choline transporter levels.

Synthesis and characterization of a fluorescent and stable nanodevice exposing folate ligands



Selective fluorescence imaging of melanoma cells



Task 4.3

Development of portable medical imaging & smart healthcare systems and devices

- **Sensors for FLASH radiotherapies** (STLab, ASM, IBSBC-CNR and INFN Catania division)
- **Smart healthcare systems and devices for pulmonary diseases** (IFT)
- **Development of a sensor for the monitoring and control of safety valves working status in mechanical ventilation systems** (IMM)
- **Translational neuroscience technologies** (IRIB)

Subtask 4.3.3: Translational neuroscience technologies / Synthesis and characterization of nano and microvesicles

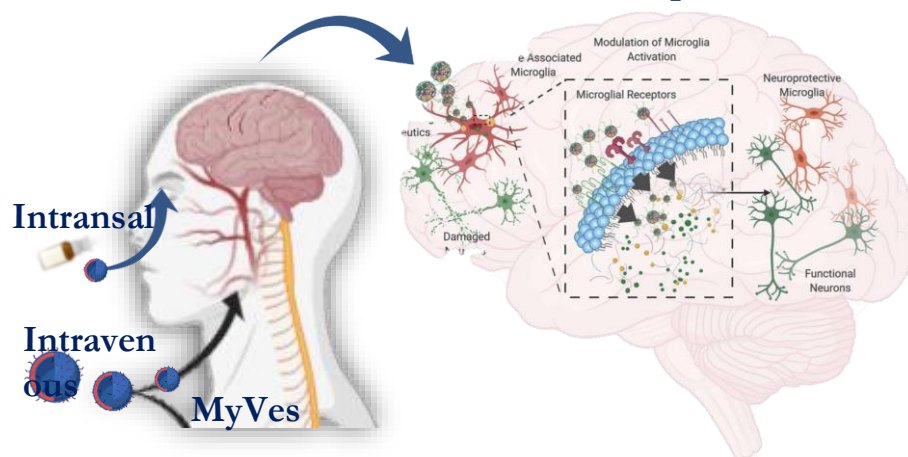
Clinical need: bio-fabrication of nanovesicles from brain tissue capable to cross BBB. The nanovesicles were produced from myelin brain, and hereafter they will be identified as Myelin nano-Vesicles (MyVes).



Product	Size (nm)	ζ-potential (mV)	PDI
MyVES	87.9±11.7	-21.4±5.7	0.583
MyVES*	88.8±18.4	-18.9±5.6	0.547
MyVESDH	90.5±14.8	-22.2±8.5	0.486

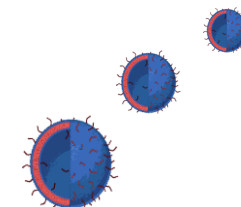
Size, Z potential and polydispersity index (PDI) values of MyVes, MyVes labeled with fluorescent probe (MyVes*) and Dexamethasone hemisuccinate MyVesDH were measured by dynamic light scattering.

•Potential economic or societal impacts.



The MyVes are cytocompatible, do not accumulate when degraded and are able to cross the BBB and specifically target the microglial cell.

Current TRL 4/5



Italian patent application n. 102020000010888, priority date 13/05/2020;

Internation patent application n. PCT/IB2021/054046, filed on 12/05/2021



By nanotherapeutic approach specifically designed to target microglia is possible to contrast **microglia-associated neuroinflammation** which can be modulated and neurodegeneration can be slowed down

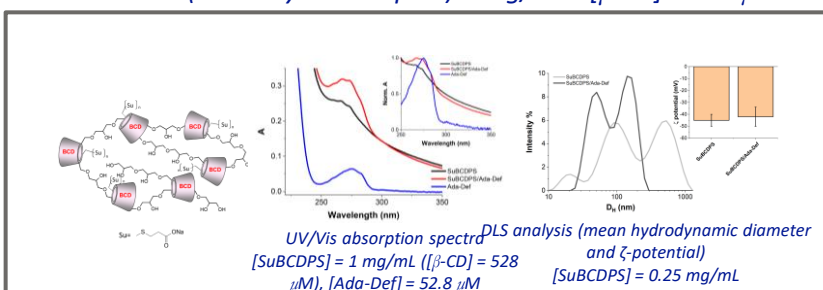
Task 4.4

Environment-related health devices

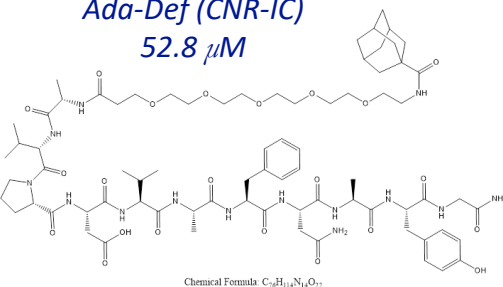
- Nanomaterials-based and antimicrobial peptides (AMPs) based antimicrobial coatings (IMM, IFT; IC, ISMN, IRIB)
- Disinfection of medical devices for use in nosocomial environments by biodegradable & biocompatible nanostructured systems with high efficiency (ICB)
- Novel spray nanoformulations for air purifying in living environments (ICB)
- Biosensors for marine ecosystems (ISP/IRIB-ME)

Anti-microbial peptides (AMP)- based for antimicrobial coatings

SuBCDPS (CarboHyde Budapest) 1 mg/mL - $[\beta\text{-CD}] = 528 \mu\text{M}$

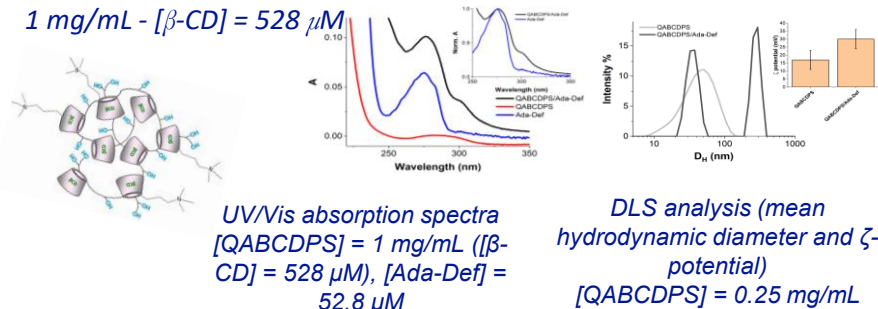


*Ada-Def (CNR-IC)
52.8 μM*

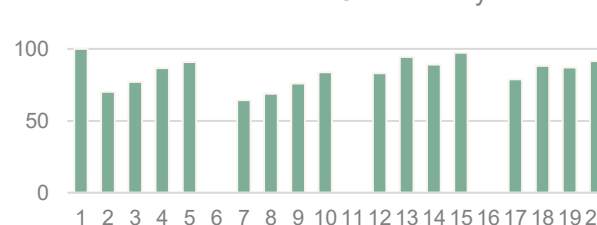


Ada-Def was complexed by both SuBCDPS (negative charged) and QABCDPS (positive charged) β -cyclodextrin polymers in a 1:10 (Ada-Def/Polymer) molar ratio (ISMN-Messina).

*QABCDPS (CarboHyde Budapest)
1 mg/mL - $[\beta\text{-CD}] = 528 \mu\text{M}$*



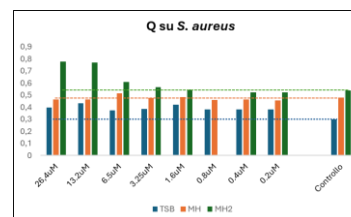
% Cell Viability after 24 h



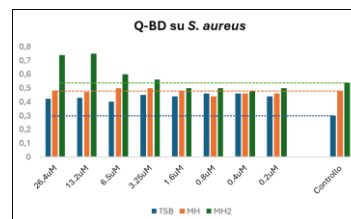
Cell viability in fibroblasts exposed to different concentrations of QABCDPS/Ada-Def and SuBCDPS/Ada-Def β -cyclodextrin polymers for 24 h, detected by MTT test (IRIB-Catania).

A= QABCDPS 2,12= 200 μM . **B**=QABCDPS/Ada-Def 7,17= 20 μM 3,13= 100 μM **C**= SuBCDPS 8,18= 10 μM 4,14=50 μM . 5,15= 10 μM 9,19= 5 μM 10,20= 1 μM

Antimicrobial activity of QABCDPS (Q) and QABCDPS/Ada-Def (Q-BD) β -cyclodextrin polymers



No inhibition of *S. aureus* growth after 24 hours was observed with both compounds at the indicated concentrations. Instead, a sharp stimulation of growth (about +50%) was observed in MH-2 medium with both compounds, which indicated that the observed activity is likely due to the nanostructure, rather than to the conjugated peptide (IRIB-Palermo).



The present **TRL status is 2** and it is **expected to afford TRL 3-4** upon formulation (i.e. hydrogel)

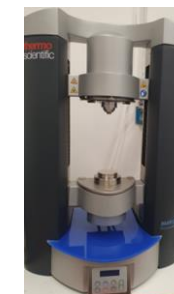
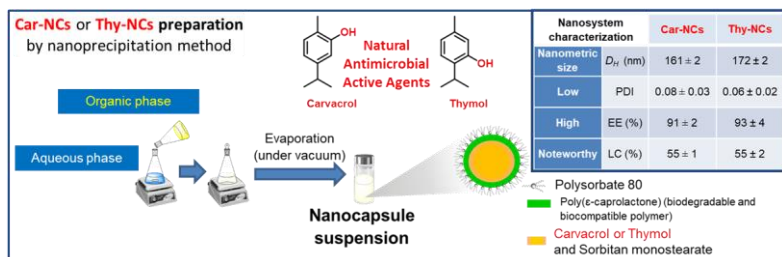
Current activity is focused on the synthesis of **AMPs based on buforin II sequences (IC)**.

Disinfection of medical devices for use in nosocomial environments by biodegradable and biocompatible nanostructured systems with high efficiency

Synthesis of Efficient Green Nanosystems: Carvacrol-loaded nanocapsules (Car-NCs) and Thymol-loaded nanocapsules (Thy-NCs).

Natural antimicrobial compounds + nanoencapsulation → higher availability (solubility, stability).

Nanoprecipitation method based on biocompatible and biodegradable polymer (Poly(ϵ -caprolactone)).



ICB Unit: Physicochemical characterization (mean hydrodynamic diameter (D_H), polydispersity index (PDI), particle size distribution, zeta potential, encapsulation efficiency (EE) and loading capacity (LC))

IFT unit: Antimicrobial tests Both act like a bacteriostatic both on gram + and gram – model bacteria; **cytotoxicity effects** The Cytotoxicity tests showed that lower concentration of thymol and carvacrol nanocapsules has **not cytotoxic effect** with a positive action on cell viability in an alveolar basal epithelial cell line A549 epithelial cell line.

- **Actual TRL 4; Final TRL expected 5-6.**
- Under evaluation the effect of Car-NCs and Thy-NCs, in combination with specific antimicrobial, against multi-resistant pathogenic bacteria. Further experiments are necessary to understand and define the action of these substances with or without particles in the mechanisms of oxidative stress and inflammation. The challenge could be improving the engineering of medical device treatments

Subtask 4.4.3: Novel spray nanoformulations for air purifying in living environment

A simple, **easy-to-use spray device to purify living environments** (public and domestic) is proposed; the final device will be safe because based on green and eco-compatible technologies and plant-derived with reduced risks of allergy and/or contact burns



Production of natural products based novel and safe spray nanoformulations able to purify the air from pathogens (Laura Siracusa, Giuseppe Granata - ICB)

Antimicrobial activity *in vitro* against the common pathogens plus *S. aureus* ATCC43300 (methicillin resistant strain) (collaboration with Salvatore Papasergi - IFT)

Safety test *in vivo* on murine tracheal and lung microbiota (collaboration with Caterina Cascio - IFT)

Implemented solubility and stability with respect to products available on the market
Moving towards TRL4 with the *in vivo* preliminary safety tests
1 possible patent

- **No similar products present on the market ; only essential oils suspended in hydro-alcoholic solutions**
- **0 conventional solvents used for the extraction; compositional analyses carried out in UPLC mode with minimum volumes of solvents required**

SPOKE 4 WP4 HEALTH PUBLICATIONS, CONFERENCES & PATENTS APPLICATIONS

- ✓ 12 Publications on International Peer Reviewed Journals
- ✓ 14 Contributions to International Conferences
- ✓ 5 Contributions to Dissemination Events
- ✓ 2 Possible patents proposals



“Multicomponent platform based on magneto-plasmonic and cyclodextrin assemblies for melanoma liquid biopsy” by *Giuseppe Nocito, Estella Rao, Mauro Manno, Riccardo Di Corato, Nadia Lampiasi, Giovanna Barbieri and Antonino Mazzaglia*, ISMN/IMM/IRIB/IBF

“Novel spray nanoformulations for air purifying living environments” by *L. Siracusa et al.* ICB

Thank you for your attention

