Nano Innovation 2021 Rome, 21-24 September Conference & Exhibition

Porous silicon microparticles for immunologic adjuvant delivery

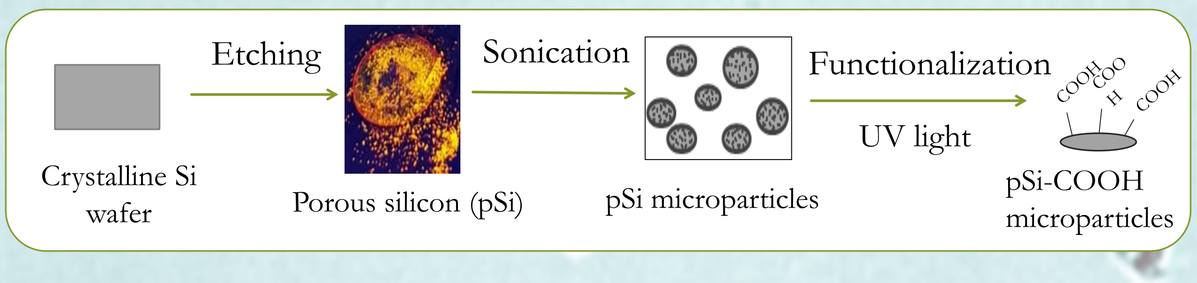
A. Sambugaro, [a] E. Chistè, [a] M. Donini, [b] M. Scarpa, [c] S. Dusi, [b] N. Daldosso [a]

[a] *Dep. of Computer Science, Fluorescence Laboratory, University of Verona, Strada le Grazie 15, 37134, Verona, Italy*
[b] *Dep. of Medicine, Division of General Pathology, University of Verona, Strada le Grazie 8, 37134, Verona, Italy*
[c] *Dep.t of Physics, Laboratory of Nanoscience, University of Trento, Via Sommarive 14, 38123, Trento, Italy*

alessia.sambugaro@univr.it

Porous Silicon particles fabrication

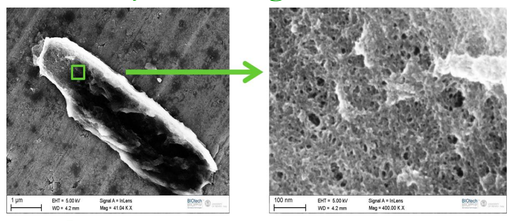
Porous silicon (pSi) is a sponge-like material produced by electrochemical etching of a crystalline silicon wafer in HF solution followed by sonication to obtain microparticles. Functionalization with carboxylic groups and storage in ethanol allows to avoid degradation and quenching of the optical properties of the pSi microparticles for years.



Properties

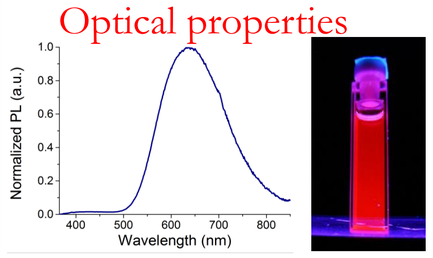
pSi particles gained a lot of interest in biomedicine because they are **porous**, **biodegradable**, **biocompatible** and show **neither toxicity nor immunogenicity**. Moreover, due to quantum confinement effect, this material is proved to be **photoluminescent** (bioimaging applications).

Porosity and large surface area



→ Drug loading and release

Optical properties

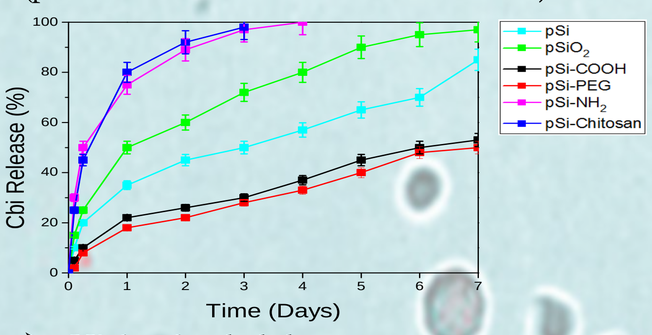


→ Bioimaging

→ Good carrier for **Nanomedicine**

Previous study

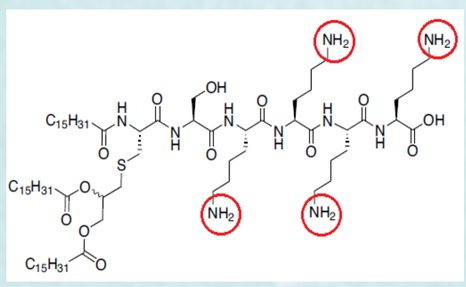
pSi-COOH microparticles loaded with **Cbi** (precursor of cobalamin vitamin B12)



- Higher loaded drug amount
- Slower drug release rate

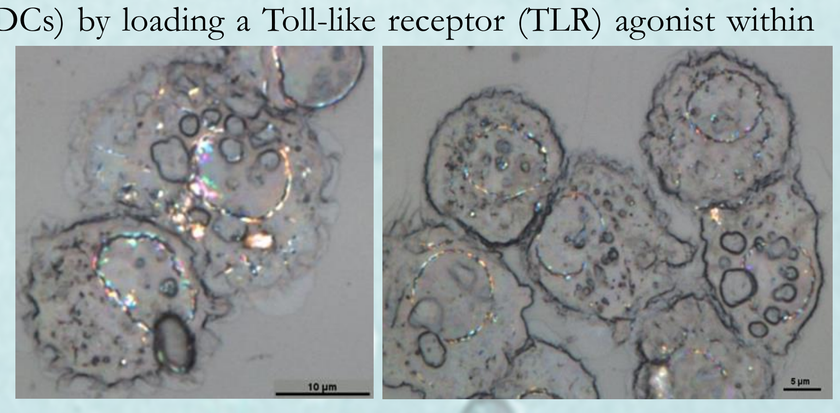
Current study: aim

Enhance immune response mediated by human dendritic cells (DCs) by loading a Toll-like receptor (TLR) agonist within carboxyl-functionalized pSi microparticles.



Pam3CSK4

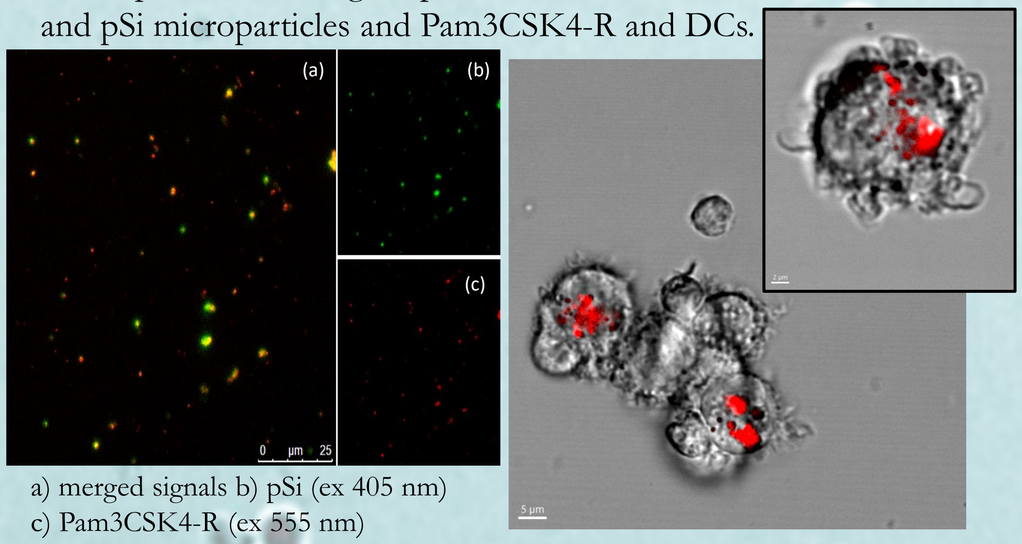
- Synthetic lipopeptide
- Fits inside pSi-COOH pores
- Multiple binding sites
- TLR 1/2 ligand
- Labeled version avaiable



Binding of Pam3CSK4 to TLR 1/2 leads to innate immune response → Study DCs stimulation promoted by loaded pSi-COOH microparticles

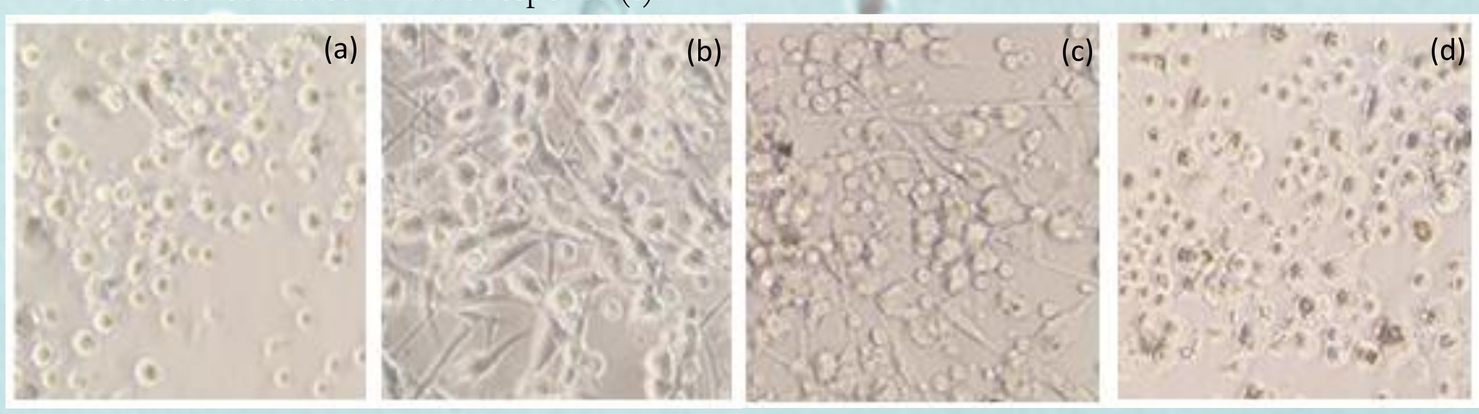
Interaction with human DCs

- **LOADING CONFIRMATION: CONFOCAL MICROSCOPY** Correspondence in signal position between Pam3CSK4-Rhodamine and pSi microparticles and Pam3CSK4-R and DCs.



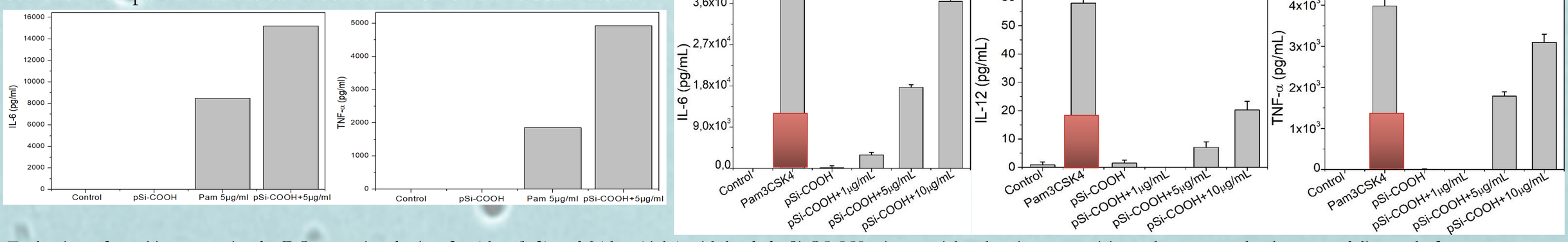
a) merged signals b) pSi (ex 405 nm) c) Pam3CSK4-R (ex 555 nm)

- **DCs ACTIVATION: OPTICAL MICROSCOPY**. The elongated shape of DCs (d) proves the activation caused by loaded pSi-COOH microparticles. As expected, pSi-COOH microparticles alone do not induce immune response (c).



DCs (a), DCs after incubation with Pam3CSK4 (b), with pSi-COOH microparticles (c) and with pSi-COOH microparticles loaded with Pam3CSK4 (d)

- **IMMUNE RESPONSE: ELISA assay**. pSi-COOH microparticles were incubated for 4 hrs (right, red columns represent the 34% encapsulation efficiency for comparison) and 24 hrs (left) with different concentrations of Pam3CSK4. Loaded microparticles show a dose-response effect, demonstrating a clear dependence between the concentration of loaded Pam3CSK4 and the amounts of secreted cytokines. Using pSi-COOH microparticles as carrier results in an enhancement of the cytokines secretion compared with the same concentration of Pam3CSK4 alone.



Evaluation of cytokines secretion by DCs upon incubation for 4 hrs (left) and 24 hrs (right) with loaded pSi-COOH microparticles showing a promising enhancement thanks to our delivery platform

Discussion & Conclusions

Loading of a TLR ligand: Pam3CSK4 was successfully loaded within pSi microparticles, as confirmed by confocal microscopy. Encapsulation efficiency was determined by fluorescence spectroscopy obtaining a value of about 34%.

Immune response: DCs were isolated and stimulated with Pam3CSK4 loaded within pSi microparticles. DCs activation was shown by means of optical microscopy and confirmed by elongation of the dendrites. Immune response was evaluated by ELISA assay, showing a promising enhancement effect resulting from the synergic interaction between pSi microparticles and Pam3CSK4 that might lead to an application in vaccination and cancer immunotherapy.

References

E. Chistè, G. Ischia, M. Scarpa and N. Daldosso, Mater. Res. Express, 2019, 6(7), 075006.

A. Ghafarinazari, M. Scarpa, G. Zoccatelli, M. Comes Franchini, E. Locatelli and N. Daldosso, RSC Adv., 2017, 7(11), 6724.

E. Chistè, A. Ghafarinazari, M. Donini, V. Cremers, J. Dendooven, C. Detavernier, D. Benati, M. Scarpa, S. Dusi, N. Daldosso, J. Mat. Chem. B, 2018, 6, 1815.

Acknowledgments

The authors acknowledge the CPT (Technological Platform Center) of the University of Verona for the access to the instrumentation