ISIS@MACH ITALIA

UKRI Science and Technology Facilities Council
ISIS Neutron and Muon Source

# Experiment Proposal

Experiment number GP2023036


**Principal investigator (*)** Dr Alessia Famengo, CNR, ITALY
**Co-investigator** Dr Francesca Visentin, CNR, ITALY
**Co-investigator** Dr Naida El Habra, National Council of Research, ITALY
**Co-investigator**
**Co-investigator**
**Co-investigator**
**Co-investigator**
**Co-investigator**
**Co-investigator**


| | | |
|---|---|---|
| **Experiment title** | Understanding the chemical composition of chemically functionalized cellulose nanofibers onto titanium dioxide substrates | |
| **MRF Instrument** | **Raman Confocal Microscope** | **Days requested:** 4 |
| **Access Route** | Direct Access | **Previous GP Number:** no |
| **Science Areas** | Biology and Bio-materials, Chemistry, Materials | **DOI:** - |
| **Sponsored Grant** | None | **Sponsor:** - |
| **Grant Title** | - | **Grant Number:** - |
| **Start Date** | - | **Finish Date:** - |
| **Similar Submission?** | - | |
| **Industrial Links** | - | |


**Non-Technical Abstract** The functionalization of inorganic surfaces such as Si or metal oxides ($SiO_2$, $TiO_2$, $Nb_2O_5$) with hydrophilic molecules and biopolymers is considered a key step for the design of biocompatible materials promoting specific cell adhesion. Nanocellulose (NC) is currently considered an important emerging biocompatible material with low toxicity potential, even if further immunological investigations are needed. We started investigating organic/inorganic composites made of nanocellulose layers deposited onto titanium dioxide surfaces as a model for prosthetic devices. We propose to use confocal Raman Microscopy for surface chemical mapping, to assess NC spatial homogeneity, the interaction with the inorganic substrate and the distribution of functional groups for future bioconjugation.


**Publications** -

---

**ISIS neutron and muon source** **IM@IT E-platform:** No

| | |
|---|---|
| **Instruments** | **Days Requested:** |
| **Access Route** | **Previous RB Number:** |
| **Science Areas** | **DOI:** |
| **Sponsored Grant** | **Sponsor:** |
| **Grant Title** | **Grant Number:** |
| **Start Date** | **Finish Date:** |
| **Similar Submission?** | |
| **Industrial Links** | |

---

# Sample record sheet

| | | | |
|---|---|---|---|
| **Principal contact** | Dr Alessia Famengo, CNR, ITALY | | |
| **MRF Instrument** | **Raman Confocal Microscope** | **Days Requested:** 4 | |
| **Special requirements:** | | | |

## SAMPLE

| | | | |
|---|---|---|---|
| **Material** | cellulose funtionalized fibers deposited onto TiO2 layers supported on Si | TiO2 thin films on Si substrates | cellulose funtionalized fibers deposited onto TiO2 layers supported on Si |
| **Formula** | - | - | - |
| **Forms** | Solid | Solid | Solid |
| **Volume** | cc | cc | cc |
| **Weight** | 3 g | 3 g | 3 g |
| **Container or substrate** | - | - | - |
| **Storage Requirements** | under dry and inert atmosphere to limit surface modification by water molecules | store in dry and inert atmosphere to limit water contamination | store in dry and inert atmosphere to limit water contamination |

## SAMPLE ENVIROMENT

| | | | |
|---|---|---|---|
| **Temperature Range** | - K | - K | - |
| **Pressure Range** | - mbar | - mbar | - mbar |
| **Magnetic field range** | - T | - T | - |
| **Standard equipment** | Do Not Know | Do Not Know | None |
| **Special equipment** | - | - | - |

## SAFETY

| | | | |
|---|---|---|---|
| **Prep lab needed** | Yes | Yes | No |
| **Sample Prep Hazards** | The sample will be prepared on the PI lab | The sample will be prepared on the PI lab | The sample will be prepared in the PI lab |
| **Special equip. reqs** | - | - | - |
| **Sensitivity to air** | No | No | No |
| **Sensitivity to vapour** | Yes | Yes | Yes |
| **Experiment Hazards** | Toxicological effects of cellulose nanofibers are not fully characterized. | The PI never perform Raman Confocal Microscopy so associated hazards are unkown | Toxicological effects of cellulose nanofibers are not fully characterized. |
| **Equipment Hazards** | - | - | - |
| **Biological hazards** | - | - | - |
| **Radioactive Hazards** | - | - | - |
| **Additional Hazards** | - | - | - |
| **Additional Details** | - | - | - |
| **Sample will be** | Removed By User | Removed By User | Removed By User |

## Science Case

### Background and Context

Nowadays, biopolymers such as carbohydrates and proteins are considered good candidates for the synthesis and design of novel biomedical materials because of their biocompatibility, high abundance, renewability and biodegradability. Among them, cellulose is increasingly studied as a renewable source of materials for biomedical applications. It is particularly prone to bioconjugation, for the presence of reactive hydroxylic (-OH) groups allowing both the interaction with biological molecules and adhesion to functional surfaces, such as those present in biomedical devices. Once converted into nanometric form, nanocellulose (NC) can be dispersed in water and processed to obtain free-standing or supported films at different thickness, with the possibility of tuning materials properties by surface engineering. NC can be produced in large quantities at a relatively low cost and it is generally classified as cellulose nanocrystals (CNC), cellulose nanofibrils (CNF) and bacterial cellulose (BC). It is currently considered as an important emerging biocompatible material with low toxicity potential, even if further immunological investigations are needed. The functionalization of inorganic surfaces such as Si or metal oxides (SiO2, TiO2, Nb2O5) with hydrophilic molecules and biopolymers has since long been considered a key step for the design of biocompatible materials promoting specific cell adhesion. In this context, we started investigating the design and characterization of NC/metal oxide composite layers exploiting tunicates as a cellulose source alternative to plants. Tunicates are invasive marine animals often discarded as waste from the aquaculture industry, able to produce an external envelope mainly composed of microcrystalline cellulose. They are a local precursor of NC, as they grow in the Lagoon of Venice. Our contribution will be devoted to the development of a multifunctional organic ad-layers grafted on inorganic protective oxide surfaces obtained via Atomic Layer Deposition (ALD) towards highly biocompatible interfaces for smart prostheses exploiting this local source of cellulose and our experience in inorganic surface engineering. This proposal will add a complementary compositional characterization of deposited CNF fibers onto TiO2 layers supported on Si, as a model for prosthesis. Specifically, chemical surface mapping can be an indication of the reactive sites for biomolecules and will guide us to improve NC chemical functionalization/deposition, TiO2 deposition conditions and post deposition treatments for surface activation. Preliminary results of such investigation are reported in the Master Thesis of Andrea Riccioni (University of Padova) https://thesis.unipd.it/handle/20.500.12608/43751 in the frame of a scientific collaboration with prof. Elisabetta Schievano (DISC-UNIPD). In particular, it is worth to note that Prof. Schievano developed an efficient nanocellulose extraction method from tunicates from Venice Lagoon. (https://www.alumniunipd.it/blog/2021/11/29/dai-tunicati-una-grande-opportunita-di-rilancio-per-la-laguna-veneta/)

### Proposed experiment

Chemical characterization of NC thin and thick films is not trivial and requires a multitechnique approach. As highlighted above, we think that Confocal Raman Microscopy, extensively used for the chemical analysis on composites (distribution of polysaccharides into cell membranes, distribution of NC into polymers, carbon nanostructures into NC network, etc) can be a complementary analytical tool to assess CNF spatial homogeneity, their interaction with the inorganic substrate and the distribution of functional groups for bioconjugation. We planned to perform surface chemical analysis via X-Ray photoelectron spectroscopy and ATR-FT-IR spectroscopy to check the presence of NC functional moieties and TiO2 film composition. Accessing to Raman Imaging facility is an opportunity to map the chemical composition on a relative large sample area with information about molecular species at the surface. Our first goal is to map the surface chemical composition of TiO2 layers deposited via ALD, identifying the presence of TiOH/TiOH2 groups and crystalline phases (rutile, anatase) and correlating these experimental findings with substrate processing prior to CNF deposition

The next step will be the chemical mapping of CNF fibers on $TiO_2$ and then compare the resulting maps with those of pristine $TiO_2$ to verify the effect of $TiO_2$ surface functional groups and crystalline phase domains on CNF spreading. Furthermore, we would like to reveal the type of interaction of CNF functional groups ($NH_3^+$, $COO^-$)with the inorganic substrate and their surface distribution as chemical maps. Confocal Raman Imaging can, in principle, evidence the effect of crystalline phase composition of $TiO_2$ domains on the CNF distribution,

**3. Summary of previous experimental proposals or characterisation**

SEM micrographs of NCC films previously recorded at ICMATE revealed the presence of NC fibers homogeneously dispersed on $TiO_2$ surfaces, as shown in figures 1, 2

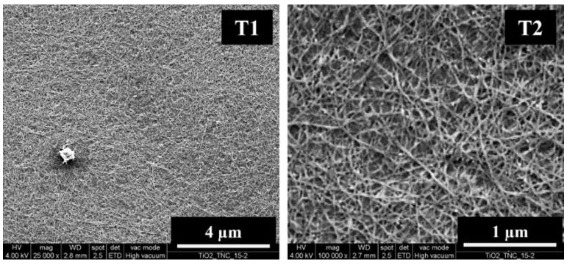


**Fig 1** SEM micrographs of $TiO_2$ surfaces covered with carboxylated CNF by spin coating

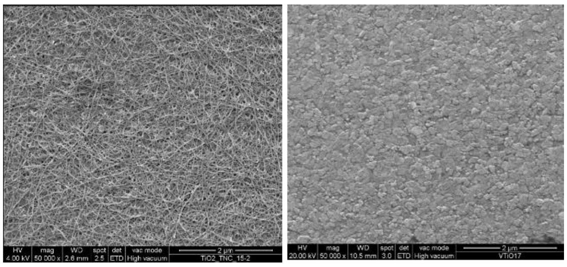

**Fig 2** SEM micrographs of $TiO_2$ surfaces covered with carboxylated CNF by spin coating: comparison with uncoated $TiO_2$ (right)

**4. Justification of experimental proposals request**

We request Confocal Raman Microscopy for 2D Raman mapping on particular surfaces made of fibrous aggregates. This is the first time we perform Raman confocal microscopy. A reasonable plan could be as follows. As indicated in the proposal template, max. 3 samples will be analysed. Their description is reported in Tabs.1, 2

| 1$^{st}$ round | n°sample | Time required (day) |
|---|---|---|
| $TiO_2$ film on Si 60-80 nm thickness | 1 | 1 |

**Table 1** Pristine $TiO_2$ substrate to be analysed prior to CNF deposition

Pristine $TiO_2$ film on Si, after being measured, will be divided into two parts, each to be covered with two different CNF formulations, as reported in table 2. This experimental part will be performed the day after at ICMATE laboratories, and the process will take approximately 2 days.

| | Type of CNF-functional group to be deposited | |
|---|---|---|
| | CNF-COO- | CNF-NH3+ |
| $TiO_2$ film on Si 60-80 nm thickness | 1 | 1 |

**Table 2** Number of CNF deposited onto $TiO_2$ samples for analysis

The two samples of table 2 can be analysed during one day. We ask for a total time of 4 days, considering 2 days of measurement and 2 days for data analysis.