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PILOT 2021 2026

DELIVERABLE REPORT

WP14 JA4 - A safe-by design platform for nanomaterials

D14.3
Fabrication of the injection system for liquids and integration with the operando system

Due date

M33

This initiative has received funding from the EU's H2020 framework program for research and innovation under grant agreement n. 101007417, NFFA-Europe Pilot Project

PROJECT DETAILS

PROJECT ACRONYM	PROJECT TITLE
NEP	Nanoscience Foundries and Fine Analysis - Europe|PILOT

GRANT AGREEMENT NO:	FUNDING SCHEME
101007417	RIA - Research and Innovation action

START DATE

01/03/2021

WORK PACKAGE DETAILS

WORK PACKAGE ID	WORK PACKAGE TITLE
WP14	A safe-by-design platform for nanomaterials

WORK PACKAGE LEADER

Dr. Hiram Castillo-Michel (ESRF)

DELIVERABLE DETAILS

DELIVERABLE ID	DELIVERABLE TITLE
D14.3	Fabrication of the injection system for liquids and integration with the operando system

DELIVERABLE DESCRIPTION

This deliverable reports on the design and construction of a prototype setup for controlled delivery of nanoparticles in a continuous flow of cell medium for subsequent integration with the operando system developed in deliverable 14.2

DUE DATE	ACTUAL SUBMISSION DATE
M33 (Month) 30/11/2023	

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NATURE

☐ R - Report

☒ P - Prototype, Demonstrator

☐ DEC - Websites, Patent filing, Press & media actions, Videos, etc

☐ O - Other

DISSEMINATION LEVEL

☐ **P - Public**

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REPORT DETAILS

ACTUAL SUBMISSION DATE
02/12/2023

NUMBER OF PAGES
11 (right-click and select "update the field")

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VERSION	DATE	AUTHOR(S)	DESCRIPTION / REASON FOR MODIFICATION	STATUS
1	16/11/2023	Barbara Sartori		Draft TUG
2	17/11/2023	Eleftheria Babaliari		Draft FORTH
3	25/11/2023	Heinz Amenitsch Barbara Sartori		Draft TUG
4	28/11/2023	Hiram Castillo		DRAFT
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D14.3 FABRICATION OF THE INJECTION SYSTEM FOR LIQUIDS AND INTEGRATION WITH THE OPERANDO SYSTEM

Introduction

In order to understand the phenomena occurring at the interfaces between contacted nanomaterials and biological materials, and to implement a safe-by-design approach for nanomaterials and to understand their life cycle in biological systems, a specific biological substrate and microfluidic system compatible with the NLOM microscopy platform in FORTH was developed (D14.2).

Initially designed as a setup for static and dynamic controlled delivery of nanoparticles (NPs) via drop casting, the system had to be modified because the biological substrate holder includes a cover to allow in-operando observation of cell cultures at the microscope. Thus, it was decided to develop a device to dispense droplets of NPs suspension in the continuous flow of cell growth medium.

FORTH's microfluidic system is composed of an air compressor and OB1 pressure controller, which are connected through tubing to the nutrient reservoir. The nutrient moves through the tubing to the flow sensor, to the bubble trap, to the ibidi µ-slide I Luer 3D microfluidic chamber containing the polydimethylsiloxane (PDMS) flat and microgrooved substrates with the cells, and finally to the waste reservoir. The µ-slide I Luer 3D microfluidic chamber is installed in the NLOM microscope for real-time imaging (see D14.2). In the described experimental set-up, the syringe pump containing the zinc oxide (ZnO) NPs was connected through a T-mixer before the bubble trap of the microfluidic system (Figure 1).

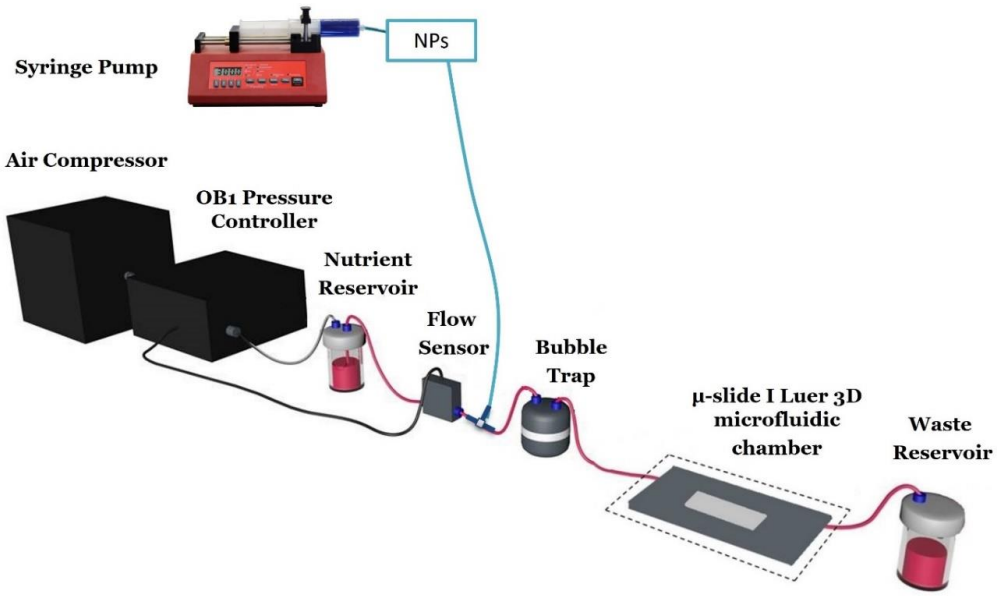

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Figure 1: Schematic representation of the integration of the injection system for liquids with the operando system.

The entire system was realized in two steps:

(i) a development set-up has been implemented and tested at TUG, which consists of an injection system with two syringe pumps, one to simulate the continuous flow infuser, available at FORTH, the second to realize the controlled injection of the NPs. This system allowed to test the software and functioning of the injection system.

(ii) the injection system has been integrated into the microfluidic set-up for the NLOM microscope allowing real-time imaging at FORTH. The entire system has been tested with real time imaging of NIH 3T3 cells exposed to ZnO NPs.

Development of the set-up

The setup is composed of a T-mixer connected on one inlet to an infuser that provides the continuous flow (i), and on the perpendicular inlet (ii) to a TSE syringe pump, which delivers NPs suspension drops being of variable size into the continuously flowing cell growth medium (Figure 2a). To observe the dispensed NPs drops after each injection, we connected the outlet of the T-mixer to a 2 mm ID, transparent polycarbonate capillary (Figure 2b). The T mixer outlet tubing inserts directly into the polycarbonate tube and is kept in place by a small piece of soft silicone tube.

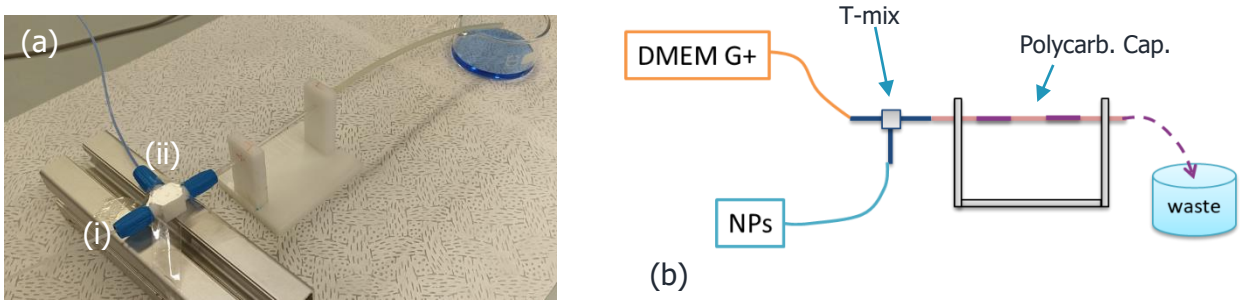

Figure 2: Picture (a) scheme (b) and of the controlled injection setup.

DMEM-HG flows continuously, at adjustable flow rates. The NPs carrier fluid is injected in shots perpendicularly to the DMEM G+ flow at defined timing. The volume of NPs dispensed in the continuous flow was chosen to obtain a dilution of 1:10 per shot (see Table 1). The flow rate can be adjusted to achieve either better diffusion in a larger volume, or more localized NPs dispensing, using a custom made in-house developed software.

T-mixer integration and software

The TSE syringe pump and T mixer are designed to be added to the existing continuous infusion setup, before the bubble trap: the T-mixer is suited for standard PTFE tubings with 1/16" outer diamater (OD) to be easily integrated. As in every microfluidic system, outmost care should be

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taken during the installation, to avoid air bubbles in the syringe. Particular care should be taken to set the correct syringe diameter. Although plastic syringes can be used, it might be a better choice to use glass body syringes, to assure accurate volume delivery.

The TSE syringe pump is controlled with an in-house developed executable software based on Labview (Figure 3). It also allows to monitor the total infused volume, which is useful in case the pump is installed e.g. inside the experimental hutch of a Large-Scale Facility (LSF), thus not always accessible. Once the setup is installed, the pump should be connected to the control PC via a RS232 straight cable (included in the setup), configured (COM port and pump address) and initialized to establish the communication with the control PC.

Various parameters such as the NPs injection volume (Target volume), the flow rate (Infuse rate), the time between each shot (Delay) and the number of shots (Times) can then be adjusted.

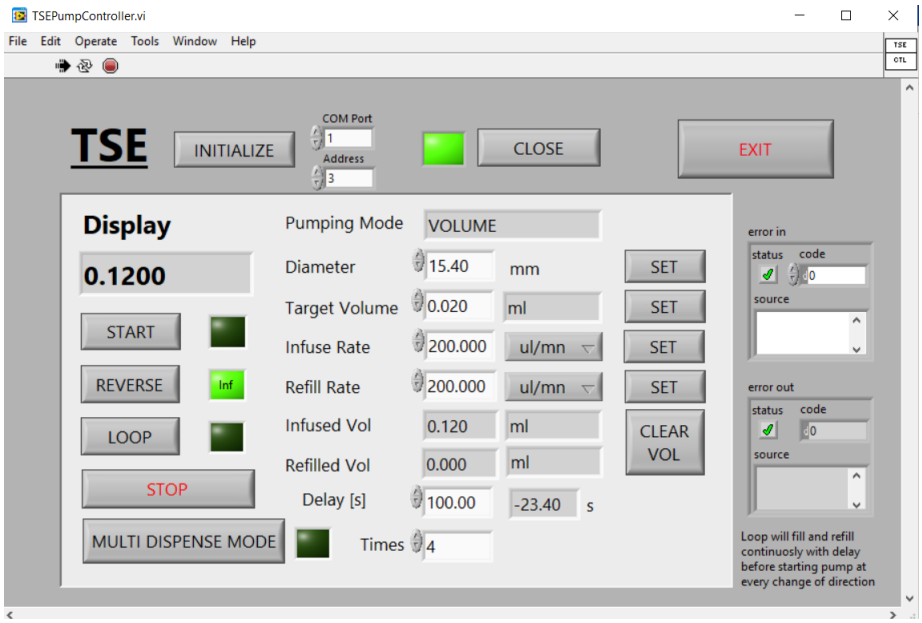

Figure 3: Screenshot of the TSE pump control interface.

Parts specifications

T mixer (Diba Omnifit® Standard Connector for 1 to 4 mm OD Tubing, 3-way, tee, Item # GZ-21939-08)

Syringe pump: TSE mod. 540060

Polycarbonate capillary OD 2 mm (KI-BEAM, ENKI, Concesio, Brescia, IT), L=100 mm.

1/16”, 0.5 mm ID PTFE tubings.

Silicone Tube connector ID = 0.76 mm, OD = 1.65 mm

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Results of set-up development

For the test experiments, the NPs suspension was simulated with Aniline blue 0.1% in H_2O. Instead of DMEM G+ cell medium, pure water was used because it has the same viscosity and allows to see better the change of color after the injection of Aniline blue.

The flow rate of both the continuous flow and of the injection shots were varied, while the time between the shots was kept constant (1 shot /60s). A summary of the parameters applied in the tests performed is reported in Table 1. Movies of the tests are available as Supporting Information.

Table 1: Parameters corresponding to the experiments performed, and corresponding video added (see supporting information).

Movie SI	CONTINUOUS FLOW RATE (µl/min)	SHOTS FLOW RATE (µl/min)	INJECTION VOLUME (µl)
Movie_S1	200	200	20
Movie_S2	200	500	20
Movie_S3	100	300	10

All three configurations proved the possibility to dispense locally a defined amount of NPs suspension. At higher injection flowrate, the Aniline suspension has less time to diffuse in the continuous flow, thus can be visualized better in the observation capillary; applying a lower continuous flowrate increased the diffusion of the Aniline blue dispensed shots. Further experiments with lower continuous flow rates, and injecting very small volumes of Aniline blue to keep the 1:10 dilution, did not allow to visualize the Aniline blue, due to excessive diffusion.

Integration and live imaging experiments

Integration of the injection system for liquids with the operando system

The injection system for liquids was integrated into FORTH's microfluidic system for live imaging observation (operando system). The microfluidic system was used to achieve a continuous flow of nutrient, and the syringe pump to realize the controlled injection of the ZnO NPs.

The analytical experimental setup of NLOM microscope was described in D14.1 (submitted) and the µ-slide I Luer 3D microfluidic chamber containing the PDMS flat and microgrooved substrates was presented in D14.2 (submitted).

Figure 4 illustrates the integration of the injection system for liquids with the operando system.

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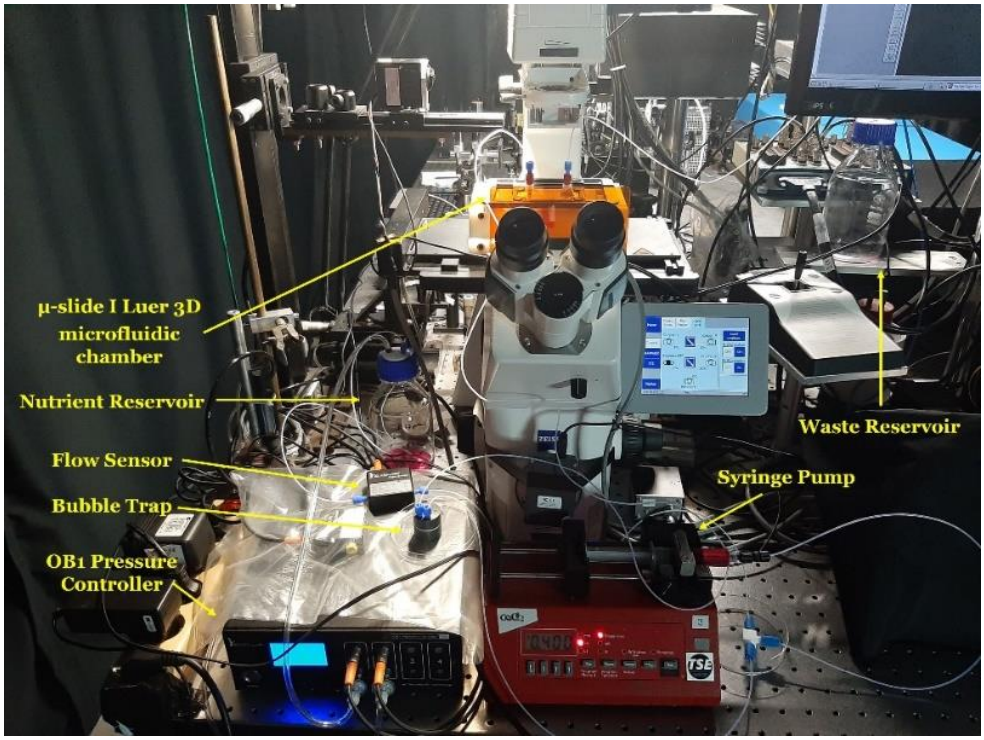

Figure 4: Illustration of the integration of the injection system for liquids with the operando system.

Observation of live NIH 3T3 cells cultured on PDMS flat and microgrooved substrates inside ibidi μ-slide I Luer 3D microfluidic chamber under ZnO NPs flow with the NLOM microscope

In accordance with D14.2 (submitted), 700000 NIH 3T3 cells/mL were cultured on the PDMS flat and microgrooved substrates inside the ibidi μ-slide I Luer 3D microfluidic chamber. NIH 3T3 cells attached strongly and proliferated well on the PDMS substrates. After 24 hours of culture, the samples were stained with the fluorescent dyes Biotracker 555 Orange Cytoplasmic Membrane dye and Hoechst (nuclei). A continuous flow (15 μL/min) of DMEM-High Glucose (HG) medium without (w/o) fetal bovine serum (FBS) w/o penstrep (PS) was used in FORTH's microfluidic system. By means of the syringe pump system, 200 μL shots of 0.0001% (1μg/ml) ZnO NPs were injected at a flow rate of 15 μL/min into the flowing nutrient medium. The delay between each shot was set to 60 sec, the duration of the NPs shots was 2 hours. The 0.0001% ZnO concentration was chosen due to the fact that was not toxic to NIH 3T3 cells as shown in D14.2 (submitted). We successfully observed the nuclei, the membrane of the live cells and ZnO NPs inside μ-slide I Luer 3D microfluidic chamber using the NLOM microscope, as depicted in Figures 5 and 6. Moreover, under the 0.0001% ZnO NPs shots, NIH 3T3 cells remained alive inside μ-slide I Luer 3D microfluidic chamber (Figures 5, 6) indicating that this concentration is not toxic to cells in agreement with the previous results presented in D14.2 (submitted).

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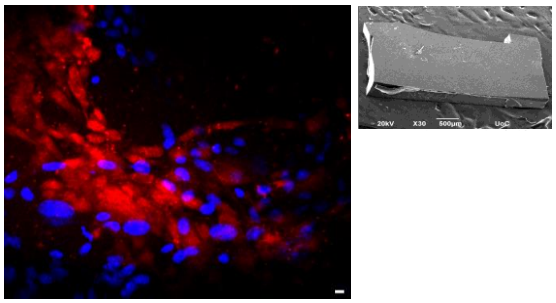
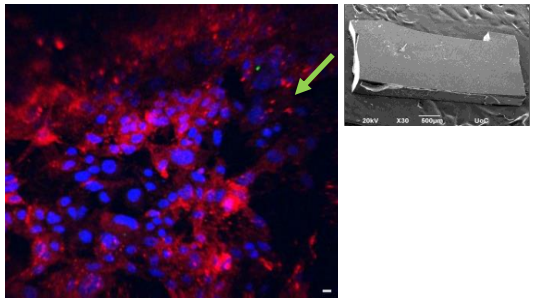
Before 0.0001% ZnO NPs shots	After 0.0001% ZnO NPs shots
	
Hoechst (nuclei): 3PEF signal Biotracker 555 Orange Cytoplasmic Membrane dye: 2PEF signal	Hoechst (nuclei): 3PEF signal Biotracker 555 Orange Cytoplasmic Membrane dye: 2PEF signal ZnO NPs: SHG signal
PDMS flat substrate	

Figure 5: Observation of live NIH 3T3 cells cultured on PDMS flat substrate inside ibidi µ-slide I Luer 3D microfluidic chamber with the NLOM microscope before and after the ZnO NPs shots. Scale bar: 10 µm. The inset SEM images depict the PDMS flat substrate.

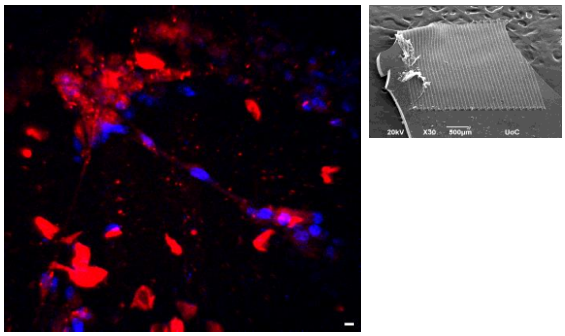
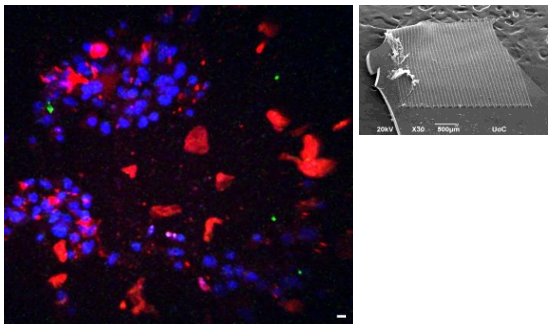
Before 0.0001% ZnO NPs shots	After 0.0001% ZnO NPs shots
	
Hoechst (nuclei): 3PEF signal Biotracker 555 Orange Cytoplasmic Membrane dye: 2PEF signal	Hoechst (nuclei): 3PEF signal Biotracker 555 Orange Cytoplasmic Membrane dye: 2PEF signal ZnO NPs: SHG signal
PDMS microgrooved substrate	

Figure 6: Observation of live NIH 3T3 cells cultured on PDMS microgrooved substrate inside ibidi µ-slide I Luer 3D microfluidic chamber with the NLOM microscope before and after the ZnO NPs shots. Scale bar: 10 µm. The inset SEM images depict the PDMS microgrooved substrate.

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Conclusions

We successfully developed an injection system for controlled delivery of liquids such as NP suspensions in a continuous fluid flow, and profitably integrated it in the operando system. Observation of the NIH 3T3 cells treated with ZnO NPs at the NLOM microscope revealed the possibility to efficiently detect the effect exerted on the cultured cells by injected ZnO NPs, both on the flat and on the microgrooved PDMS surface. We demonstrated as well that the controlled injection of NPs shots did not disturb the flow rate of the cell medium, thus avoiding any harmful effect on cells functionality. Moreover, the experiments performed with the controlled flow in-operando integrated setup demonstrated that ZnO NPs shots at the chosen concentration (0.0001%) seem to be not toxic to NIH 3T3 cells cultured on PDMS flat and microgrooved substrates inside the ibidi µ-slide I Luer 3D microfluidic chamber for a short-time period, in agreement with previously reported results.

Supplementary information

SI movies of the test setup, showing injection of different volumes of aniline blue in a continuous flow of pure water

Movie S1 – 20µl shots at 200 µl/min

Movie S2 – 20µl shots at 500 µl/min

Movie S3– 10µl shots at 300 µl/min

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