ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2023023

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Experiment title	Investigation on the inner structure of porous and filled gelatin microparticles
MRF Instrument	**FIB-SEM GAIA 3**
Access Route	Direct Access
Science Areas	Biology and Bio-materials, Chemistry
Sponsored Grant	Yes
Grant Title	MERMAID *Project*–Next Generation EU
Start Date	-
Similar Submission?	-
Industrial Links	-

Days requested: 1
Previous GP Number: No
DOI: No
Sponsor: Other
Grant Number: -
Finish Date: -

Non-Technical Abstract

Gelatin is a protein-based biopolymer obtained from the partial hydrolysis of collagen, the main constituent of mammalians' connective tissues. Gelatin microparticles (GM) have been proven to be a relevant and versatile platform for a range of biomedical applications, including drug delivery and tissue engineering, due to gelatin's high biocompatibility. FIB-SEM TESCAN GAIA 3 in combination with ultramicrotome and with chemically-enhanced contrast, will enable the imagining of the inner structure of dry GM by precisely cutting the microparticles without damaging it. The FIB option opens up unique opportunities for our research, that has strongly relied upon SEM results up to now, but without the possibility of looking to the inner structure. We are planning to investigate about three different microparticles type (both filled and porous).

Publications

ISIS neutron and muon source

IM@IT E-platform: No

Instruments	ZOOM, IMAT
Access Route	
Science Areas	
Sponsored Grant	
Grant Title	
Start Date	
Similar Submission?	
Industrial Links	

Days Requested: 3
Previous RB Number:
DOI:
Sponsor:
Grant Number:
Finish Date:

Sample record sheet

Principal contact Miss Giulia Mugnaini, University of Florence, ITALY
MRF Instrument **FIB-SEM GAIA 3** **Days Requested:** 1
Special requirements:

SAMPLE

Material	Gelatin, glutaraldehyde, glyceraldehyde	-	-
Formula	CHNO	-	-
Forms	Solid		
Volume	ml		
Weight	100 mg		
Container or substrate	Eppendorf	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	Room Temperature - K	-	-
Pressure Range	Room Pressure - mbar	-	-
Magnetic field range	None - T	-	-
Standard equipment	None	-	-
Special equipment	No	-	-

SAFETY

Prep lab needed	No	-	-
Sample Prep Hazards	No	-	-
Special equip. reqs	No	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	No	-	-
Equipment Hazards	-	-	-
Biological hazards	No	-	-
Radioactive Hazards	No	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Disposed of by instrument scientist	-	-

Science Case
Investigation on the inner structure of porous and filled gelatin microparticles

1. Background and Context

Gelatin is a protein-based biopolymer obtained from the partial hydrolysis of collagen, which is the main constituent of mammalians' connective tissues. Gelatin microparticles (GM) have been proven to be a relevant and versatile platform for a wide range of biomedical applications, including drug delivery and tissue engineering, due to gelatin's high biocompatibility. Notably, GM are promising candidates for the development of 3D microscaffolds for cell cultures, in which the cell-microparticle assembly preserves the stemness properties and enhances the differentiation cells abilities. Emulsification technique is a well-established procedure for the fabrication of gelatin-based microparticles with controlled morphological properties: the use of a single water-in-oil (W/O) emulsion results in the formation of filled GM, whereas following a more complex double oil-in-water-in-oil (O/W/O) emulsion porous GM can be prepared. Gelatin's reversible *sol-gel* transition represents the main drawback of the use of this biopolymer, especially in biomedical applications taking place in aqueous environments at physiological temperature (~37 °C); hence a chemical cross-linking treatment is usually necessary to stabilise gelatin-based systems. Widely used chemical bifunctional cross-linkers are glutaraldehyde and glyceraldehyde. Nevertheless, the application of GM as an artificial extracellular matrix (ECM) requires a proper design, since ECM regulates cellular behaviours and, at the same time, provides mechanical support. The incorporation into microparticles' inner and external surfaces of cell-recognition sites, which could guide cell attachment, proliferation and differentiation, is an utmost important topic and in this framework fits one of the purposes of the 'Targeting the immunometabolic role of MerTK in the microenvironment of intrahepatic cholangiocarcinoma -MERMAID- financed by University of Florence through the European Union – NextGenerationEU' grant, who financially supports our work. Our main goal is the realisation and physicochemical characterisation of cross-linked gelatin-based microparticles with a filled or porous structure, whose surface is functionalized with β-D-Lactose derivatives as cell-recognition sites, for the development of biocompatible platform for tissue engineering. Aiming at that, the study of the internal morphology and porosities represents a crucial point since these features deeply influence drug, nutrient, growth factors and cells diffusion, encapsulation, availability and release in the microenvironement.

2. Proposed experiment

Owing to the double emulsion protocol, it is reasonable to assume that the internal structure of porous microparticles is characterised by interconnected porosities in the range of micrometers. On the other hand, filled GM should not display an inner porosity, apart from the one generated by gelatin hydrogel network, which appears in the nano and sub-nano scale. Despite FE-SEM equipment which only allows the external microparticle's surface to be displayed, FIB-SEM TESCAN GAIA 3 instrumentation, possibly in combination with ultramicrotome and with chemically-enhanced contrast, available through *ISIS@MACH ITALIA* enables the imagining of the inner structure of dry GM by precisely cutting the microparticles without damaging it. Furthemore, these measurements coupled with neutron tomography and small angle neutron scattering, performed respectively with IMAT and Zoom instrumentation available at ISIS Neutron and Muon Source, on swollen state microparticles will provide a global characterization of the inner and external structure at a multi-level scales of the microparticles at both dry and swollen states. Previous confocal Raman 3D volume maps (**Fig. 1i**) collected on dry microparticles in our laboratories by means of confocal Raman microscopy displayed an alternation of dark and light areas, which correspond respectively to void and fill areas.

suggesting the presence of interconnected pores in the inner structure of GM. The acquired micrographs will be analysed with the software ImageJ and pore's dimension will be evaluated by superimposing a circle equivalent to the pore's area; the resulting pore's size will be used to obtain size distributions, which will be fitted according to mathematical function.

3. Summary of previous experimental proposals or characterisation

Porous GM were prepared according to a double O/W/O emulsion, by systematically varying the stirring speed and gelatin/surfactant ratio, in order to study the possibility to tuning the morphological features. They were characterised in our laboratories by means of a multi-techniques approach, including investigation on both dry and swollen state. The morphology of dry GM, analysed by means of FE-SEM (**Fig. 1ii**), demonstrates a strong dependence of the microparticle's shape and radius on the preparation protocols, enabling us to define some general guidelines for the preparation of porous gelatin microparticles with tailored morphologies. The stability of cross-linked GM was thoroughly assessed with dissolution tests, confirming the possibility to extend GM's stability from hours up to days according to the cross-linkers used (namely glutaraldehyde and glyceraldehyde). X-ray scattering experiments (**Fig. 1iii**) performed on swollen uncross-linked and cross-linked GM revealed that typical nanoscale structure of the gelatin hydrogel network was preserved upon cross-linking with an average mesh size (~3 nm) in agreement with values reported in the literature. Filled GM were fabricated following the single W/O emulsion method and two different hydrophobic phase (sunflower oil and olive oil) were independently investigated. FE-SEM confirmed the ability of the selected protocol to fabricate non porous microparticles with a quite smooth surface (**Fig. 1iv**).

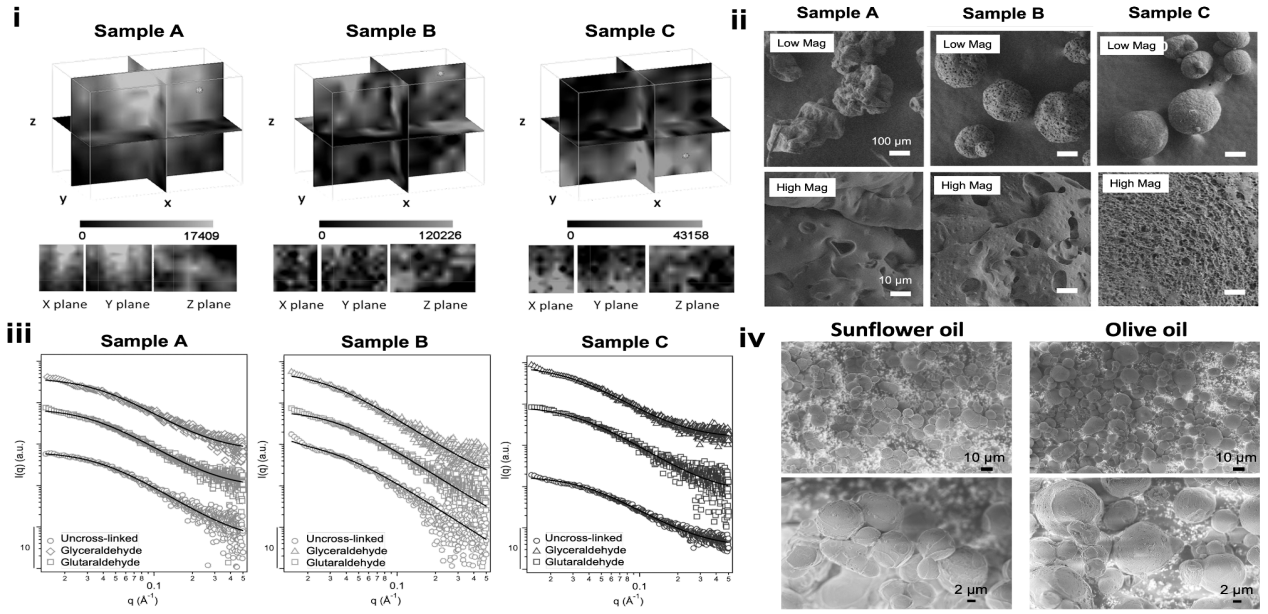

Fig. 1. Confocal Raman volume maps and *x*, *y*, and *z* slices (i), FE-SEM micrographs (i) and SAXS curves (ii) of porous GM samples prepared with different stirring speed and gelatin/surfactant ratio [reprint from DOI: 10.1021/acs.langmuir.1c01508]; FE-SEM micrographs of filled GM prepared with different hydrophobic phases.

4. Justification of experimental proposals request

The FIB option opens up unique opportunities for our research, that has strongly relied upon SEM results up to now, but without the possibility of looking to the inner structure. We are planning to investigate about three different microparticles type (both filled and porous) for a total number of samples of 6 and a working time of 1 day.