ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2024016

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Experiment title	Investigation of the architecture of agarose-based hydrogels prepared by controlled rate of cooling - AGAROCOOL	
MRF Instrument	**SAXS GISAXS**	**Days requested:** 2
Access Route	Direct Access	**Previous GP Number:** GP2023083
Science Areas	Biology and Bio-materials	**DOI:** -
Sponsored Grant	Yes	**Sponsor:** Regional found
Grant Title	UnderstandiNg cardiac FIBROSIS using unprecedented dissipative substrates - NoFIBROSIS	**Grant Number:** D40-microgrants23_SACCO
Start Date	01/04/2023	**Finish Date:** 31/03/2025
Similar Submission?	-	
Industrial Links	-	

Non-Technical Abstract

Extracellular matrices (ECMs) and, in a broader sense, living tissues can be described as complex biopolymer-based networks that are endowed with special physical and mechanical properties. The cells that make up the tissues can sense this biophysical environment and transduce the resulting external physical information into intracellular biochemical signals through a process known as 'mechanotransduction'. Our research group is interested in recapitulating this physical information in ECM mimics in the form of hydrogels and using them as a platform to study important mechanotransduction processes in cells. The aim of AGAROCOOL is to investigate the role that the methylation pattern of the biopolymer agarose, the temperature of cooling of its solutions after autoclaving and the ionic strength of the medium play in the formation of three-dimensional hydrogels. Ultra/SAXS experiments will enable a breakthrough in the study of the architecture of these networks.

Publications -

ISIS neutron and muon source **E-platform:** No

Instruments	**Days Requested:**
Access Route	**Previous RB Number:**
Science Areas	**DOI:**
Sponsored Grant	**Sponsor:**
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Sample record sheet

Principal contact Dr Pasquale Sacco, Università degli Studi di Trieste, ITALY
MRF Instrument **SAXS GISAXS** **Days Requested:** 2
Special requirements:

SAMPLE

Material	The hydrogels will be assembled using agaroses with different chemical composition (methylation degree) in the presence of different salts to modulate the ionic strength	-	-
Formula	Biopolymers formed by alternating D-galactose and 3,6-anhydro-L-galactopyranose linked by α-(1→3) and β-(1→4) glycosidic bonds	-	-
Forms	Friable powder		
Volume	cc		
Weight	500 mg		
Container or substrate	Plastic falcon	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	298 - 310 K	-	-
Pressure Range	1000 - 1000 mbar	-	-
Magnetic field range	- T	-	-
Standard equipment	Water Bath	-	-
Special equipment	-	-	-

SAFETY

Prep lab needed	Yes	-	-
Sample Prep Hazards	-	-	-
Special equip. reqs	To dissolve agaroses in deionized water supplemented with salts we need autoclave or microwave	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	-	-	-
Equipment Hazards	The preparation of hydrogels will involve the use of health hazard salt such as ammonium sulfate	-	-
Biological hazards	The preparation of hydrogels will involve the use of an oxidazing salt (magnesium perchlorate)	-	-

Radioactive Hazards	None radioactive hazards associated with the samples	-	-
Additional Hazards	Other	-	-
Additional Details	The preparation of hydrogels will involve the use of health hazard salt such as ammonium sulfate	-	-
Sample will be	Removed By User	-	-

1. Background and Context

Extracellular matrices (ECMs) and, more broadly, living tissues can be described as complex biopolymer-based networks endowed with particular physical/mechanical properties. The cells that make up the tissues can sense this biophysical milieu and convert the resulting external physical information into intracellular biochemical signals through a process called mechanotransduction.[1] ECM mimics in the form of hydrogels are urgently needed to recapitulate the correct ECM composition and mechanics and use them to understand various biochemical aspects in both cell biology and pathology. Recently, we have focused our attention on agarose, a linear polysaccharide derived from red algae. It is a thermoresponsive biopolymer that dissolves completely in water when heated and forms a wall-to-wall hydrogel when cooled to room temperature. Our research group has recently shown for the first time that the controlled cooling of agarose or the presence of salts, which determine a different ionic strength, are crucial for the surface-core distribution of the biopolymer in the hydrogel network and that this influences the nanomechanical properties of the surface, the mechanical behaviour of the bulk and the response of the cells.[2,3] These results were obtained as part of a funded research project in which the applicant is PI.

2. Proposed experiment

The aim of this proposal is to detail differences in the architecture of the hydrogel network at the micro-/nanoscale when we vary the **chemical composition** of the agarose, the **temperature** of curing and the **ionic strength** of the system. The possibility of gaining access to the instruments of MRFs would be a unique opportunity to expand the knowledge of the relationship between the architecture of agarose-based hydrogels and the associated behaviour. Ultra-/SAXS will be the best technique for this type of analysis as it is a powerful tool to reveal the structure of biomaterials and allows samples to be analysed in their wet state without the need for special sample preparation procedures. The scattering curves will be analysed considering a hierarchic model where the proper mesh-size of the 3D network, the interaction of the polymer with the solvent and the presence of large scale inhomogeneities in the hydrogel are combined and explicitly considered to describe the overall structure from the nano- to the micro-scale.[4] The curve fitting will be performed with the help of the local contact (E. Fratini) using an ad-hoc routine implemented in SasView. The results will be used to complete a drafted manuscript to be published in a leading journal in the sector.

3. Summary of previous experimental proposals or characterisation

We have already thoroughly investigated the effects of the rate of cooling upon heating (quenching) of a agarose sample with low methylation content on the mechanical properties of the resulting hydrogels.[2] The experimental protocol consists of dissolving 1% w/V agarose by autoclaving (*i.e.* 121 °C) and then controlling cooling-steps (85, 60 and 42 °C) before gelling at room T (Figure 1).

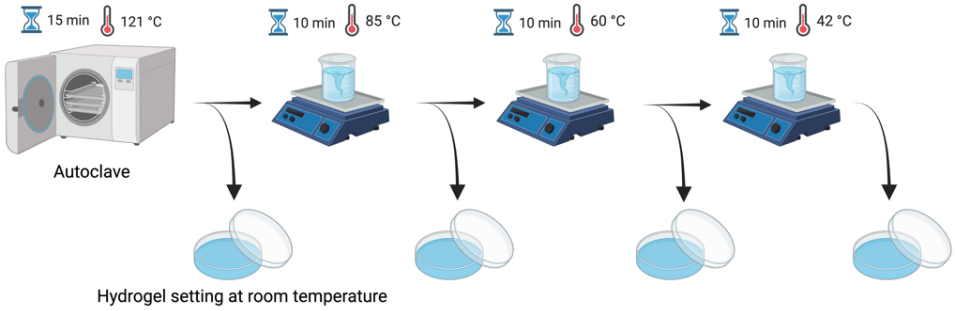

Figure 1. Cartoon recapitulating the experimental steps to form agarose hydrogels, which involves controlled cooling steps before the hydrogel forms at room temperature.

The bulk properties of the hydrogels were characterized by uniaxial compression and rheological tests, which showed that these networks relax the stress rapidly and exhibit similar stiffness, but have a linear elasticity that increases with decreasing cooling rate. A combination of SEM and confocal microscopy has revealed differences in the heterogeneity of the network architecture of the hydrogel depending on the cooling step. Unfortunately, these techniques are limited to submicron scale (confocal) or require solvent removal (SEM), thus giving questionable results. Furthermore, we have shown that the ionic strength generated by different alkali salts neighbouring in the Hofmeister series has an influence on the setting and mechanical performance of agarose hydrogels. When the ionic strength is increased from 0 to 1 M, the network becomes increasingly softer.[3]

4. Justification of experimental time requested

Ultra-/SAXS is an advanced tool able to elucidate the architecture and homogeneity of the network of agarose hydrogels, which is a key aspect in the field of mechanotransduction. We will test three agarose samples with well-identified chemical composition, which we have previously investigated by NMR analyses.[5] These agaroses will be used for the preparation of hydrogels using the temperature-assisted gelation protocol described above. The effect of two different cooling steps will be investigated. In addition, the effect of ionic strength in the range 0 – 1 M is investigated using the following salts NaCl, $(NH_4)_2SO_4$ and $Mg(ClO_4)_2$, which are relatively far down the Hofmeister series. We assume that a total of 18 samples (3 agaroses with different chemical composition x 2 cooling rate x 3 salts = 18) need to be analyzed. The estimated time for the entire setup and analysis, taking into account the ultra/SAXS measurements on the Xeuss 3HR to cover the dimensional range from half nanometer to few micrometres, would be 2 days in total (18 samples x (1.5 h USAXS + 0.7 h SAXS at 1800 mm + 0.3 h SAXS at 450 mm)) + 3 h for calibration/samples loading/detector distance change (total = 48 h).

References

[1] *Nature* **2020** *5847822*, *584*, 535
[2] *Adv. Healthc. Mater.* **2023**, *12*, 2300973
[3] *Gels (Basel, Switzerland)* **2024**, *10*, 94
[4] *ACS Appl. Mater. Interfaces* **2022**, *14*, 7471
[5] *Adv. Funct. Mater.* **2023**, *33*, 2307224