Experimental report

06/11/2018

Proposal: **9-13-777** **Council:** 4/2018

Title: Characterisation of protein nanosheets assembled at the air-water interfaces for cell adhesion and culture

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: **Ali ZARBAKHSH**

Experimental team: Dexu KONG
Pengfei LIU
Ali ZARBAKHSH
Julien GAUTROT

Local contacts: Armando MAESTRO

Samples: Pentafluorobenzoyl chloride surfactants
Beta Casein

Instrument	Requested days	Allocated days	From	To
FIGARO	3	2	17/09/2018	19/09/2018

Abstract:

Cell adhesion and proliferation at the surface of liquids such as oil droplets is a surprising phenomenon as it is typically accepted in the field of bioengineering that cells require solid substrates to adhere and to exert mechanical forces required for their spreading and proliferation. Our laboratory recently reported such observations and uncovered that such phenomenon was mediated by a nanoscale (15-20 nm) mechanically strong protein film (nanosheet) assembled at the interface between the two liquids. However, little is known of the morphology and structure of such protein nanosheet and the impact of such structure on their mechanics, and in turn cell behaviour. This project aims to explore the structure of protein nanosheets assembled at analogous air-water interfaces, in situ.

1 PRINCIPAL INVESTIGATOR
Name and institution of the Principal Investigator
Dr A Zarbakhsh Department of Chemistry Queen Mary University of London UNITED KINGDOM

2 EXPERIMENT DETAILS	
Experiment: 9-13-777	
Title: Characterisation of protein nanosheets assembled at the air-water interfaces for cell adhesion and culture	
Instrument: FIGARO	
Dates scheduled: 17/09/2018 to 19/09/2018	No. Days allocated: 2
Date of experimental report: 17/09/2018 to 19/09/2018	

3 EXPERIMENT OBJECTIVES

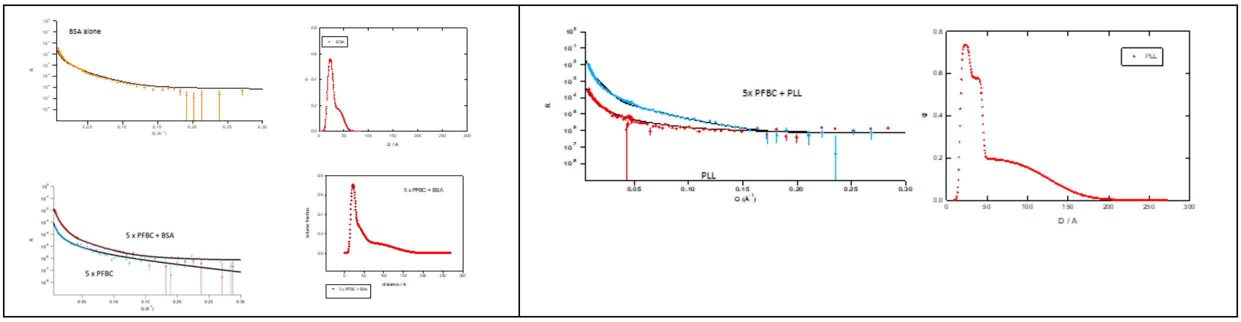
Cell adhesion and proliferation at the surface of liquids such as oil droplets is a surprising phenomenon as it is typically accepted in the field of bioengineering that cells require solid substrates to adhere and to exert mechanical forces required for their spreading and proliferation. Our laboratory recently reported such observations and uncovered that such phenomenon was mediated by a nanoscale (15- 20 nm) mechanically strong protein film (nanosheet) assembled at the interface between the two liquids. However, little is known of the morphology and structure of such protein nanosheet and the impact of such structure on their mechanics, and in turn cell behaviour. This project aims to explore the structure of protein nanosheets assembled at analogous air-water interfaces, in situ.

Biomaterials applied to stem cell-based technologies have transformed the fields of cell biology (design of novel in vitro models)1 and tissue engineering and medicine (stem cell isolation, culture, delivery and scaffolds for tissue repair)2 . In this context, biomaterials require the design of their biochemistry, as well as their physical and mechanical properties3 , to mimic the inherent ability of extra-cellular matrix (ECM) proteins to sustain cell adhesion, regulate cell function and phenotype4 . Recent data suggest that bulk solid substrates are not necessary to promote cell adhesion, growth and fat regulation: a number of reports demonstrated that cell phenotype is directed by the nano-tomicroscale biochemical and physico-chemical parameters of their environment, rather than bulk mechanical properties5-7 . This concept further suggests that cells can be cultured on materials with very weak bulk mechanical properties, but displaying strong nanoscale mechanics. The development of cell culture at liquid-liquid interfaces is set to transform stem-cell based technologies, allowing the formation of cell sheets and the design of emulsion-based 3D bioreactors. Underlying such technologies, nanosheets assembled from proteins and polymers, at oil-water interfaces should enable cell adhesion, (binding of integrin receptors) and provide a nanoscale (interfacial) mechanical environment supporting cell-mediated forces. The designs of such nanosheets require that we fully understand the physico-chemical properties of such protein/polymer assemblies at fluid interfaces, in particular how the chemical composition of nanosheets impact on their morphology (thickness, hydration, incorporation of surfactants) and mechanical properties.

4 EXPERIMENT REPORT

In this experiment we made an attempt to answer: • The relationship between the surfactant adsorbed amount (area per molecule (A2)) and the nanosheet (surfactants/ Albumin (BSA)) composition at the interface. • What is the conformation of the BSA? • What is the BSA content of the nanosheet layer? • Is the interfacial film is a result of surface complex formation of surfactants / BSA or BSA layer adsorption or both?.

The compositions of nanosheet at the air/water interface were resolved in the presence of BSA and PLL. BSA and PLL concentrations (0.04 mg/ml) were charetresised first for two contrasts of the aqueous sub-phase (D2O & CMAir). The structures of spread monolayer were determined. Pentafluorobenzoyl chloride surfactants (PFBC, C7ClF5O, Nb = 2.36 x 10-6 Å -2)) were then spread from chloroform and conformation of the final interfacial films were determined. The fits are shown by solid lines below. The volume fractions of the interfacial films are also shown.



BSA	12/2 A	22/2	
BSA+PFBC x 5	12/2	18/2	82/35
PLL+PFBC x 5	12/2	16/2	85/34
BSA	42%	82%	Water
BSA+PFBC x 5	26%	67%	89%
PLL+PFBC x 5	7%	27%	75%

5 LIKELY OUTCOMEs FROM EXPERIMENT

Please indicate what the experiment is likely to lead to by putting an 'x' next to one or more of the possible outcomes below.

Likely outcome	
Journal publication	x
Data for thesis	x
Follow-up experiment at ILL	-
Follow-up experiment at another facility	x
Other	x
No outcome anticipated	-

6 SUGGESTIONS FOR IMPROVEMENTS TO YOUR EXPERIMENT, EQUIPMENT OR THE FACILITY

NA