School of Engineering

Preliminary Report on MSc Project

Please tick appropriate project:

ENG5059P (MSc)	✔

Student Name	Nuruddin Bahar
Student Matriculation Number	2373100B
Degree programme	MSc (Biomedical Engineering)
Working Title of Project	Design of novel mechano-responsive hydrogels for drug delivery and gene therapy
Name of Supervisor(s)	Manuel Salmeron-Sanchez, Oana Dobre (First) & Thomas Franke (Second)
Academic year	2018-2019

Introduction

The development of hydrogel-based biomaterials represents a promising approach to generating new strategies for tissue engineering and regenerative medicine[1]. Stem cell fate has been linked to the mechanical properties of their underlying substrate, affecting mechanoreceptors and ultimately leading to downstream biological response[2]. Most of the studies have focused on establishing a connection between substrate stiffness and its corresponding cellular response. This project aims at understanding the mechanisms by which human mesenchymal stem cells (hMSCs) remodel 3-D poly(ethylene-glycol)-laminin (PEG-LM) hydrogels and the influence that the hydrogel mechanical and structural properties have on cell behaviour. Earlier report[3] made a few important findings – (1) bulk shear modulus of hydrogels was found to be strongly dependent on the level of applied normal force (2) since cells are known to apply pN-nN tractions on their surroundings, they are highly likely to "feel" the low-level force shear elastic modulus (3) cells are able to modify the mechanical properties of their surrounding environment, both at bulk and micro scale level. However, it was also found that it is incomplete to describe the cellular biomechanics by a single shear elastic modulus and the correlation of cell behaviour with only bulk rheological parameters could not be determined clearly.

The project involves three broad components (1) 3-D PEG-LM hydrogel preparation (Figure 1 and Figure 2) (2) culture and incorporation of MSCs to the hydrogels (3) systematic mechanical characterization of hydrogels using bulk rheology and microrheology* techniques (Figure 3). Laminins (LMs) are high molecular weight heterotrimeric extracellular matrix (ECM) glycoproteins present in the basement membrane of most tissues[4] (Figure 1 (a)). It has various isoforms that possess different affinities to several growth factors (GFs) that have been shown to bind to its heparin binding domains (HBDs)[5]. It has been found that GFs are most effective when bound to a carrier molecule like laminin compared to its soluble form[6]. This property of laminin combined with tunable mechanical properties of PEG hydrogels provide a good basis to conduct research on ECM-like biomaterials with varied stiffness and degradability, in addition to proven biocompatibility. PEG-LM based hydrogels are semi-synthetic hydrogels which are polymerized via chemical cross-linking using chain-growth polymerization reactions[7] (either photopolymerization using a photoinitiator or via Michael addition reaction). In photopolymerization, hydrogel networks are formed from the functional PEG molecules and the reaction is initiated from reactive centres, such as free radicals, generated from the photocleavage of initiator molecules (like Irgacure 2959®)[8]. Michael-type addition cross-linking prevents the use of photoinitiator and UV light, but instead require a nucleophilic buffering reagent,[9] (e.g. TEA or HEPES[10]) to facilitate the addition reaction (Figure 1(b)). In current work, to produce a covalently cross-linked hydrogel network, laminin is combined with 4 arm acrylate PEG (4-Ac-PEG) to obtain PEG-LM hydrogel[11]. In both types of reactions, PEG-LM is cross-linked with a thiol-flanked PEG-macromer to produce a non-degradable hydrogel and an enzyme-degradable peptide VPM[12], which is cleavable by collagenase (matrix metalloprotease-1 (MMP-1)) enzymes released by hMSCs[13] and is a measure to control the degradability of the hydrogel[14] (Figure 1(c)). Incorporating hMSCs into the hydrogel and measuring its stiffness overtime will help in future studies based on optimizing the hydrogel parameters for use as scaffolds to aid in osteogenesis and vasculogenesis of hMSCs[15]. Final hydrogels are prepared using PDMS molds with specific geometrical dimensions, as shown in Figure 2. Ultimately, the stiffness of these hydrogels is measured using rheological studies based on bulk rheology and microrheology, as shown in Figure 3.

Aims/Objectives

- To verify and compare results and rheological data from previous report[3]
- To check the stiffness of PEG-LM hydrogels having different degradabilities using bulk and microrheology by
  - Varying 4-arm Ac : VPM ratio (2:1, 1:1, 1:2) in 5% (w/v) PEG-LM hydrogel using hMSCs
  - Changing the concentration of laminin in the hydrogel at the same 4-arm Ac-PEG : VPM ratio of 2:1
  - Varying the % (w/v) of 4-arm Ac-PEG in the hydrogel (3.5%, 8.5%, 10%, 12%, 15%)

Resources required

For hydrogel preparation: Human recombinant LM521 (MW = 762 KDa, Biolamina ®), Ac-PEG-NHS (MW = 2 KDa, Laysan Bio, Inc. ®), phosphate-buffered saline (PBS), sodium bicarbonate ($NaHCO_3$) solution (pH 8.5, 1M), 4-Ac-PEG (MW = 10 KDa, Laysan Bio, Inc. ®), HS-PEG-SH (MW = 2 KDa, Creative PEGWorks ®), Irgagure 2959 ®, PDMS molds (diameter 17.2 mm and thickness 2 mm for bulk rheology, diameter 5 mm and thickness 2 mm for microrheology, giving a gel volume of approximately 500 µl and 50 µl, respectively), UV light (OmniCure ® Series 1500, Excelitas Technologies Ltd.), VPM (MW = 1.7 KDa, GenScript ®), polystyrene (PSS) particles (1 µl /ml, particle radius =0.77 µm, 2.5 µl per gel). *For cell-laden hydrogel preparation:* Human MSCs from bone marrow (PromoCell GmbH) and culture media specific to hMSCs (DMEM (FBS), Promocell media, DMSO) in addition to the resources mentioned in hydrogel preparation. *For bulk rheology:* Parallel plate, stress-controlled rheometer (MCR302, Anton Paar ®), prepared hydrogels with dimensions D_{bulk} and h as shown in Figure 2(c), 15 mm and 25 mm upper parallel plates. *For microrheology:* Passive video-tracking microrheology apparatus including an inverted

* or microscale characterization using a nano-indentor, based on instrument availability

microscope, with objective lens (100X, 1.3 Numerical Aperture, oil immersion, Zeiss, Plan-Neofluar), metal-oxide semiconductor camera (Dalsa Genie HM640 GigE) and a custom made particle tracking software (LabVIEW (National Instruments)), prepared hydrogels with dimensions D_μ and h as shown in Figure 2(c).

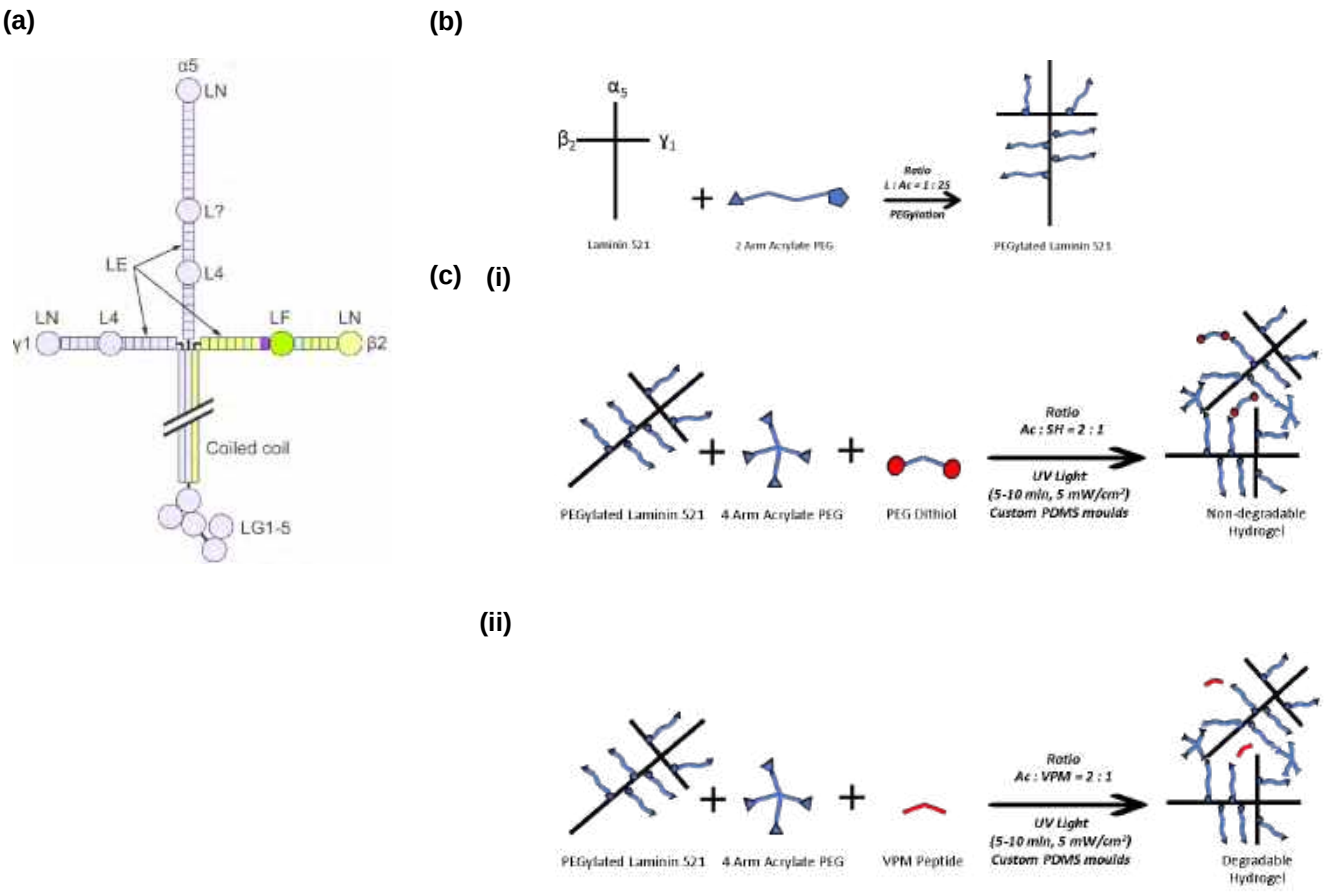
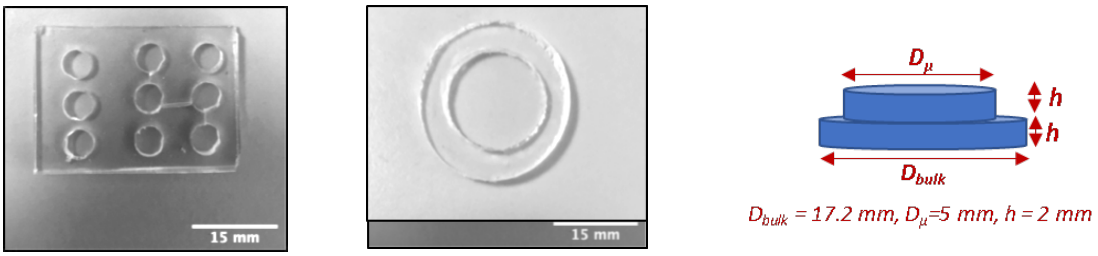
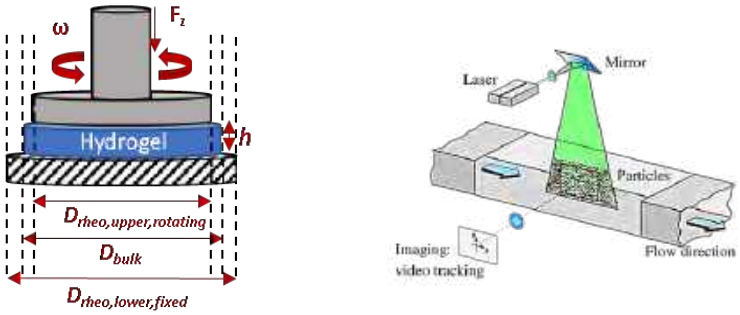


Figure 1. Mechanism of PEG-LM hydrogel preparation (a) Domain structure of laminin-521 (LM)[16] **(b)** PEGylation of LM **(c)** Photopolymerization reaction for **(i)** non-degradable and **(ii)** degradable hydrogels.



(a) (b) (c)

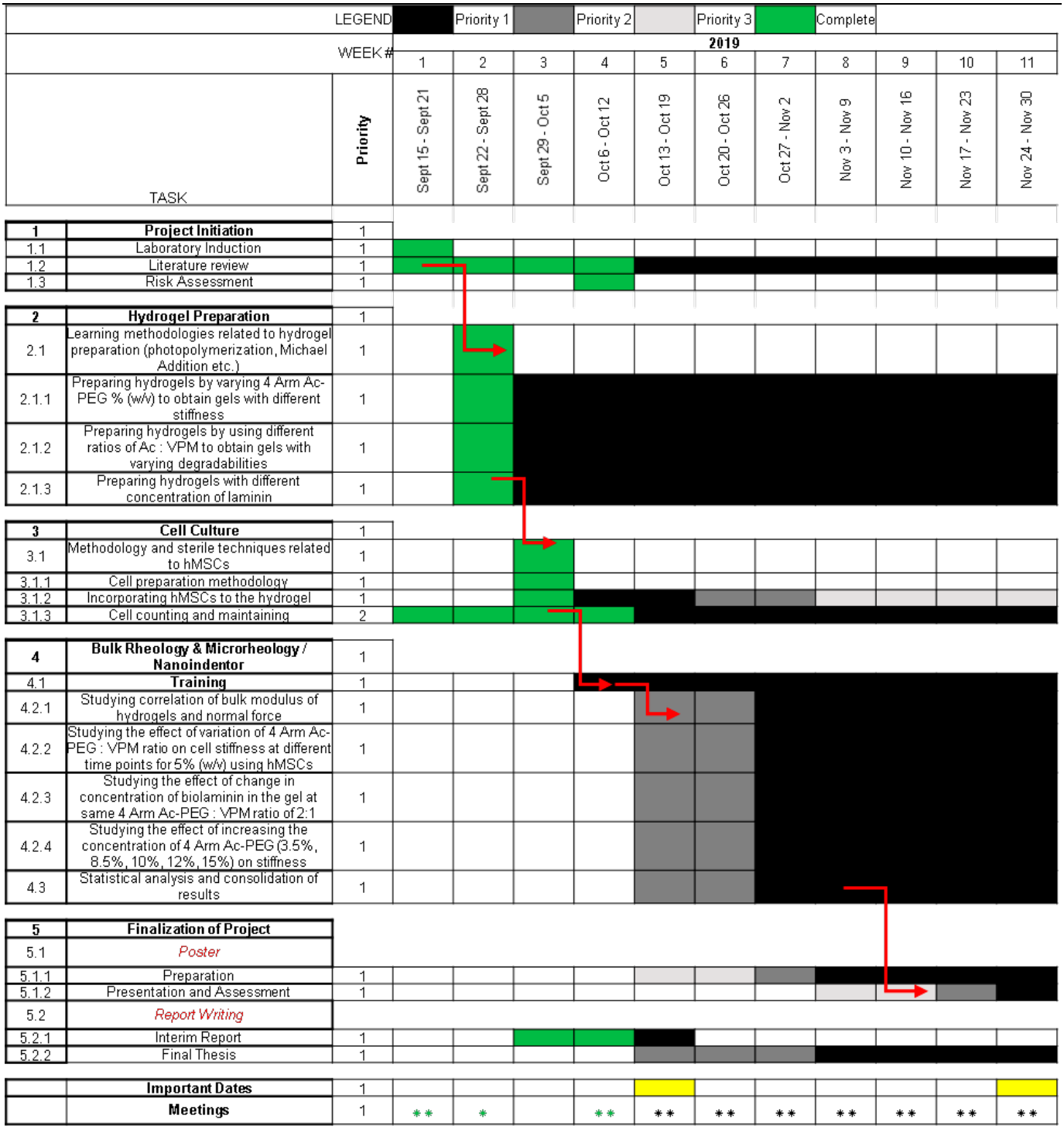
Figure 2. PEG-LM hydrogel preparation in lab (a) Custom PDMS molds **(b)** Prepared PEG-LM hydrogels (larger for bulk rheology and smaller for microrheology) **(c)** Dimensions of hydrogels.



(a) **(b)**

Figure 3. Methods to measure hydrogel stiffness (a) Bulk rheology **(b)** Microrheology[17].

GANTT chart


LEGEND
Priority 1
Priority 2
Priority 3
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WEEK #
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TASK
Priority
Sept 15 - Sept 21
Sept 22 - Sept 28
Sept 29 - Oct 5
Oct 6 - Oct 12
Oct 13 - Oct 19
Oct 20 - Oct 26
Oct 27 - Nov 2
Nov 3 - Nov 9
Nov 10 - Nov 16
Nov 17 - Nov 23
Nov 24 - Nov 30
1 Project Initiation 1
1.1 Laboratory Induction 1
1.2 Literature review 1
1.3 Risk Assessment 1
2 Hydrogel Preparation 1
2.1 Learning methodologies related to hydrogel preparation (photopolymerization, Michael Addition etc.) 1
2.1.1 Preparing hydrogels by varying 4 Arm Ac-PEG % (w/v) to obtain gels with different stiffness 1
2.1.2 Preparing hydrogels by using different ratios of Ac : VPM to obtain gels with varying degradabilities 1
2.1.3 Preparing hydrogels with different concentration of laminin 1
3 Cell Culture 1
3.1 Methodology and sterile techniques related to hMSCs 1
3.1.1 Cell preparation methodology 1
3.1.2 Incorporating hMSCs to the hydrogel 1
3.1.3 Cell counting and maintaining 2
4 Bulk Rheology & Microrheology / Nanoindentor 1
4.1 Training 1
4.2.1 Studying correlation of bulk modulus of hydrogels and normal force 1
4.2.2 Studying the effect of variation of 4 Arm Ac-PEG : VPM ratio on cell stiffness at different time points for 5% (w/v) using hMSCs 1
4.2.3 Studying the effect of change in concentration of biolaminin in the gel at same 4 Arm Ac-PEG : VPM ratio of 2:1 1
4.2.4 Studying the effect of increasing the concentration of 4 Arm Ac-PEG (3.5%, 8.5%, 10%, 12%, 15%) on stiffness 1
4.3 Statistical analysis and consolidation of results 1
5 Finalization of Project
5.1 Poster
5.1.1 Preparation 1
5.1.2 Presentation and Assessment 1
5.2 Report Writing
5.2.1 Interim Report 1
5.2.2 Final Thesis 1
Important Dates 1
Meetings 1
** * ** ** ** ** ** ** **

Risk assessment

When carrying out project work, it is vital to take all appropriate steps to prevent injury, ill health, damage or loss arising from the work.

Details of potential risks and controls can be found in the main safety and other specialist manuals on the School website:

https://www.gla.ac.uk/schools/engineering/students/

Together with your supervisor, you should complete a risk assessment using the template (https://webapps.eng.gla.ac.uk/tools/risk/), indicating the risks (e.g. mechanical, optical, chemical, biochemical or other) associated with your project and the control measures you will use to mitigate these risks. Example risk assessments can be viewed on the School safety webpages (under the Risk Assessment and Teaching Tabs). In essence, a risk assessment involves you thinking about what could possibly go wrong and then working out how to minimize both the chances and consequences of it going wrong.

Finally, any injury, dangerous occurrence, existing or potential hazard that arises during your project must be reported to the safety co-ordinator (Douglas Irons) as well as your supervisor.

Completed online risk assessment.
(1) Photopolymerization of PEG-LM Hydrogels (Single user – Chemical)
(2) Culturing human mesenchymal stromal cells (hMSCs) (Single user – Bio)

Please give the name and contact details of the person – next of kin - the university should contact in case of you are involved in an accident during your project.

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Please give the name and contact details of your usual doctor – your GP.

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