Insert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
Hazard Group 1

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/080

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological agents (BAs) must be carried out in advance of work commencing. A key requirement of The Control of Substances Hazardous to Health Regulations (COSHH) is to assess the risks associated with any work activity involving the use of biological materials may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTIONS OF PART B, AND ALL OF PART C. WHERE HAZARD GROUP 2 BIOLOGICAL MATERIAL IS INTENDED TO BE USED THE RISK ASSESSMENT MUST BE REVIEWED BY THE DEPT/SCHOOL BIOLOGICAL SAFETY ADVISOR AND EXPLICIT APPROVAL IS ALSO REQUIRED FROM THE UNIVERSITY BIOLOGICAL SAFETY OFFICER. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH, SAFETY & ENVIRONMENT UNIT FOR REVIEW VIA YOUR DEPARTMENTAL BIOLOGICAL SAFETY ADVISOR.
3. It is the responsibility of the Principal Investigator/Supervisor to ensure compliance to these requirements and that this risk assessment remains valid.
4. This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities**.

Date Submitted:	01/07/2014	Date Approved:	
Version Number:		Supersedes *(insert version number if applicable)*	

PART A: Please provide the following general information:

School/Department			
Centre for Biological Engineering (CBE)			
Title of Project			
Heterotopic Ossification Tissue Engineering – Endothelial Model			
Project Reference Number:	REQ14124		
Person responsible for this work (*Principle Investigator*)			
Name:	Yang Liu	Position:	Senior Lecturer of Healthcare Engineering
Department:	Mechanical and Manufacture Engineering	University School:	Wolfson School
Person conducting this assessment			
Name:	Owen Davies	Position:	Research Associate
Department:	CBE	Date Risk Assessment Undertaken:	01/07/2014
Proposed Project Start Date:	02/06/2014	Proposed Project End Date:	01/12/2015

Review History: *required at least once a year or immediately following any significant change to the project. Significant revisions must be detailed on a revision form. The person responsible must ensure that this RA remains valid.*

	Review 1	Review 2	Review 3	Review 4	Review 5
Due Date					
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

This provides a useful background for the reviewer and reader. It need only be brief and should provide an overview of the scientific goals.

The project will utilise a mouse cardiac endothelial cell (MCEC) line and culture with vascular endothelial growth factor (VEGF) in a 3D collagen gel to promote angiogenesis. Following angiogenesis the construct will be subjected to physical damage using a surgical blade and transferred to a dynamic perfusion chamber where it will be introduced to a number of inflammation factors that includes tumour necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), interleukins-1 and -6 (IL-1, IL-6), insulin-like growth factor-1 (IGF-1) and fibroblast growth factor (FGF). The effect of these inflammatory factors on the phenotype of endothelial cells will be examined to determine whether they have induced reversion to a stem-like state.

A1.2 Description of the Experimental Procedures

Describe laboratory procedures to be used and highlight any non-standard laboratory operations. This may need cross reference to supporting documentation i.e. protocols.

Culture of MCEC in DMEM and FBS. MCECs will be expanded in culture and cryopreserved in liquid nitrogen to produce a cell bank. MCECs will be cultured in three-dimensional collagen hydrogels to produce tissue engineered models of angiogenesis. A dynamic perfusion culture chamber will be used to examine the effect of cytokines involved in inflammation and wound healing (TNF-α), TGF-β, PDGFs, IL-1, IL-6, IGF-1, and FGF) on the phenotype of the vascular endothelium. Cell phenotype will be examined using immunocytochemistry, RT-PCR, microscopy and flow cytometry. All procedures will be conducted in accordance with the laboratory quality management system (QMS) requirements, good cell culture practice, good aseptic technique, the local code of practice (COP) and the Loughborough University Biological Safety Policy.

Preparation of culture medium:
Dulbecco's Modified Eagle's Medium (DMEM) with 5% FBS, penicillin (100U/mL)/streptomycin (100µg/mL), HEPES (10mmol/L)

Preparation of differentiation medium:
DMEM with 2% FBS, penicillin (100U/mL)/streptomycin (100µg/mL), VEGF

Receiving and storing cells:
Cells will be shipped by VH Bio, Gateshead in a liquid nitrogen transport container. Cells will be stored in the liquid nitrogen cryostorage unit – See SOPs 005, 008, 013, 031, 032

Thawing and growth:
Thaw frozen vial in a 37°C water bath. Contents will be transferred to a 75cm^2 culture flask. Initiate expansion by incubating at 37°C, 5% CO_2. Cells will be expanded until ~80% confluence is achieved and cell viability and cell counts performed. Confluent cultures will be subcultured into further 75cm^2 culture flasks using trypsin: EDTA – see SOPs 006, 017, 024, 025

Cell counting:
100µL cell suspension will be mixed with an equal volume of Trypan blue and transferred to a haemocytometer. 4 large squares will be counted, an average taken, which is multiplied by the dilution factor and then by 10,000 to give the number of cells per mL – see SOPs 029, 033, 034

Collagen hydrogel/MCEC constructs:
0.3mL x10 minimal essential medium (MEM) is added to 2.6mL type-I rat-tail collagen (dissolved in 0.1M acetic acid) and NaOH used to neutralise the solution for polymerisation. After neutralisation $12x10^6$ MCECs suspended in 0.1mL growth medium will be added to the collagen solution, and the collagen solution pipetted into a glass chamber

PART B: Please provide information in one or more of the following sections, as appropriate. Only sections which you complete should be submitted:

Section 1: *micro-organisms (prions, viruses, bacteria, fungi, parasites in ACDP category 2 and pathogens controlled by the Department for the Environment, Food and Rural Affairs).* ***[Work with ACDP category 3 and 4 pathogens is not currently permitted in the University.]***

Section 2: *cell cultures, tissues, blood, body fluids or excreta*

Section 3: *plants and plant material*

Section 4: *animals and animal tissues*

SECTION 2: CELL CULTURES, TISSUES, BLOOD, BODY FLUIDS OR EXCRETA

B2.1 HAZARD & RISK IDENTIFICATION : NATURE OF CELLS, TISSUES OR BODY FLUIDS

*This information gives an indication of the **potential** harm that the biological material may cause*

B2.1.1 List all cells or tissues to be used. *For cells indicate if primary, continuous or finite.*

Indicate in the adjacent box if Not Relevant (N/R)			
Cell or tissue type and ID	Organ Source	Species	From where will it be obtained?
Mouse cardiac endothelial cells (MCEC) (continuous)	heart	Mouse	VH Bio, Gateshead

B2.1.2 List all blood, body fluids or excreta to be used

Indicate in the adjacent box if Not Relevant (N/R)		
Material type	Species	From where will it be obtained?
Foetal Bovine Serum	Cow	Invitrogen

B2.1.3 Has any material listed in section B2.1.1 been genetically modified in any way?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete Genetically Modified Organisms (GMO) Risk Assessment Form	

B2.1.4 Will material be screened for infectious agents? (if from a cell culture collection answer B2.1.6 instead)

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, provide details of the types of screening and agents screened for: Mycoplasma Testing	

B2.1.5 Will any clinical history (if relevant) be provided with this material?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	N/R
If yes give details:	
If yes, will a policy of rejection of samples from diseased patients be adopted? Explain	
If yes, how will the information be disseminated in the course of the project?	
If yes, will this information be anonomised?	

B2.1.6 If obtained from a cell culture collection, is safety information provided?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, summarise here: Safety information is provided by manufacturer (VH Bio)	

B2.1.7 Has any of the material listed in section B2.1.1 been identified in the list of cross-contaminated or misidentified cell lines, available on HPA website
(http://www.hpacultures.org.uk/media/E50/3B/Cell_Line_Cross_Contaminations_v6_0.pdf

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, provide details of the route of provenance back to the originator of the cell line, together with a Certificate of Analysis; identifying the methods used to qualify the cell type.	

B2.2 RISK TO HUMANS

B2.2.1 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected*

Cell type and ID	Risk Category	Justification for Selection
MCEC	None	Originally obtained from microvascular neonatal mouse cardiac endothelium, classified as hazard group 1
		If none proceed to section B2.2.4

****see The Managing the risks in laboratories and healthcare premises – available at***
http://www.hse.gov.uk/biosafety/biologagents.pdf

B2.2.2 If low, medium or high risk (section B2.2.1), name and classify the Biological Agents this material could be infected with. List the biological agents and indicate the ACDP hazard group classification*

Name of Agent	Classification

****see The Approved List of Biological Agents – available on the Health & Safety website or***
http://www.hse.gov.uk/pubns/misc208.pdf.

B2.2.3 Describe the route(s) of infection (in humans) for these adventitious agents (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
				X
Details:				

B2.2.4 Are there any other biological hazards (other than adventitious infectious risk) associated with the materials e.g. aggressive tumourogenic cell lines

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No

If Yes, describe:

B2.3 HUMANS AT INCREASED RISK OF INFECTION

B2.3.1 Do any of the agents listed in section 2.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers)?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, Occupational Health must be consulted:	

B2.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B2.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify the cells and the conditions these will grow: MCECs will be cultured at 37°C, DMEM (Eagle) with 5% FBS	

B2.4.2 If culturing, will CD4+ cells be present. Describe what cells and for how long these cultures will be allowed to grow

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.4.3 If culturing, what is the maximum volume of culture grown?

Indicate in the adjacent box if Not Relevant (N/R)	
Per Flask **MCEC:** $1x10^6$ cells per $75cm^2$ flask 8mL liquid volume per $75cm^2$ flask	Per experiment Maximum of 10 $75cm^2$ culture flasks, 80mL maximum liquid volume

B2.4.4 Will the cells be manipulated in any way that could result in a concentration of any adventitious biological agent present?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.5 WORKING WITH MATERIAL DONATED BY YOURSELF OR COLLEAGUES : Persons MUST NOT work with their own cells.

B2.5.1 Will any cells be donated by persons working in or has access to the lab?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain what precautions are to be taken to prevent that person being exposed to the cells:	
If yes, where will this material be collected:	
If yes, provide justification for not using a safer source:	
If yes, how will confidentiality be assured:	
If yes, has Ethics Committee approval been obtained:	

B2.6 ENVIRONMENTAL CONSIDERATIONS:

B2.6.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.6.2 Will there be any other environmental risks?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.7 OTHER HAZARDS

B2.7.1 Are there any other hazards associated with this work? For example, hazardous chemicals (especially carcinogens, mutagens, substances toxic to reproduction, cytotoxins), cryogenic gases, ionising radiation.

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes

If yes, identify these:

Trypan blue – essential for manual cell counting – will be used and disposed of in accordance with CBE COP and SOP029 **safe handling and disposal of Trypan blue*77*

Liquid nitrogen – cell storage system, will be used and disposed of in accordance with CBE COP and SOP013 **use and maintenance of liquid nitrogen stores**

Surgical blade – required to inflict physical damage to tissue engineered endothelium, will be disposed of in accordance with CBE COP and SOP003 **disposal of biological (healthcare) waste**

Flow cytometry – required to profile cell surface markers, will be maintained and operated in accordance with SOP138 **maintenance and operation procedures of the Guava HTS flow cytometer**

Cell proliferation assessment – MTT assay required to characterise cell proliferation of MCECs, will be performed in accordance with SOP098 **cell proliferation assessment using MTT assay**

Inflammatory factors –TNF-α, TGF-β, PDGF required to investigate the role of inflammation in heterotopic ossification, will be used in BSC, which will be used and maintained in accordance with SOP009 **Use and Maintenance of Herasafe KS Class II BSC**

Sharps – haemocytometer and glass slides/coverslips (for information see C1.2.1)

Immunohistochemistry – use of formalin as a fixative (toxic)

If yes, have these been risk assessed and any necessary approval obtained?

Use of cryogenic stores will be carried out only by an authorised person in accordance with SOP013 and using the appropriate PPE

Trypan blue used according to SOP029

DMSO will be used as a cryopreservative in accordance with COSHH form CBE41 and SOP031

Formalin will be used in accordance with COSHH form 69

B2.7.2 Are there any conditions associated with the hazards described in B2.7.1 that require special attention in Section C of this risk assessment? For example, material incompatibilities with disinfectants such as Virkon or hazardous product decomposition associated with high temperatures (ie autoclaving).

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, provide details and ensure that appropriate control measures are addressed in Section C:	

SECTION 4: ANIMALS AND ANIMAL TISSUES

B4.1 HAZARD AND RISK IDENTIFICATION: NATURE OF ANIMALS OR TISSUE

*This information gives an indication of the **potential** harm that the biological material may cause*

B4.1.1 List all animals or animal tissues to be used

Species	Sex	Source	Anatomical Site	Origin or geographical source
BD Collagen Type-I	Unknown	Connective tissue	Rat tail	Commercial supplier: First Link, Birmingham
Foetal Bovine Serum	Unknown	Cow	Foetus	Commercial supplier SEFC Biosciences, UK

B4.1.2 Is the animal or tissue/body fluid to be worked with infected or to be infected?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete Section 1 of this form FBS contains material of animal origin. The material contains no hazardous or toxic substances. The material is supplied by a major commercial company and packaged securely. FBS is liquid and stored at -20°C. Rat tail collagen has been tested for hepatitis B antigen and HIV-1 antibody. All preparations are quality controlled by SDS-PAGE and tested for the presence of hazardous substances. Rat tail collagen is supplied in liquid form and delivered in secure packaging. The material is frozen at 2-8°C on receipt.	

B4.1.3 Is a carcinogen, drug or other substance to be administered to the animal(s) or present in the tissue?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete the appropriate Chemical COSHH Assessment	

B4.1.4 Have the investigators that will be performing the work on animals obtained the appropriate Home Office Licence?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If No, consult the H&S Office.	

B4.1.5 Have Standard Operating Procedures (SOPs) for the proposed work been approved?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If No, consult the H&S Office. If Yes attach the signed approval.	

B4.2 RISK TO HUMANS

B4.2.1 The disease(s) caused to humans
Describe the type and severity of effects or disease(s) on human health (including infection, allergy, bites and scratches)

Name of animal/animal tissue	Type	Severity
Type-I rat-tail collagen	Product is filtered with a 0.2µm membrane filter and tested for bacteria, fungi and mycoplasma	Potential contact irritant
FBS	Produce is pre-treated with gamma irradiation. Likelihood that it contains substances hazardous to health is low	Potential contact irritant

B4.2.2 What is the likelihood of infection of this material? INDICATE as None, Low Risk, Medium Risk, High Risk, Known Infected

Name of agent	Risk Category	Justification for Selection
Type-I rat-tail collagen	None. Animal proteins may cause potential contact irritation	Well authenticated/characterised product from a commercial source. Potential infectivity eliminated/reduced – product filtered through a 0.2µm membrane and been tested for bacterial, fungal and mycoplasma contamination
FBS	None. Animal proteins may cause potential contact irritation	Well authenticated/characterised product from a commercial source. Potential infectivity reduced/eliminated by gamma irradiation
		If none proceed to section B4.3

B4.2.3 Describe the routes of that the effects described in section B4.2.1 are transmitted (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
X	X			
Details:				

B4.3 HUMANS AT INCREASED RISK OF INFECTION

B4.3.1 Do any of the agents listed in section B4.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers, workers repeatedly handling or multiply dosing animals)?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, Occupational Health must be consulted:	

B4.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B4.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

If yes, complete Section 2 of this form:

B4.4.2 How many animals will be used?

Indicate in the adjacent box if Not Relevant (N/R)	N/R

B4.5 ENVIRONMENTAL CONSIDERATIONS: Risk to other animals

B4.5.1 Will there be any risk other animals?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe:	

B4.5.2 Will there be any other environmental risks?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe:	

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

The risk of exposure must be prevented or adequately controlled to minimise the chance of harm arising. COSHH Regulations require minimum containment measures for laboratories handling organisms from the different ACDP hazard groups (http://www.hse.gov.uk/pubns/misc208.pdf)
The hazard group number typically indicates the level of containment (includes physical measures & working practices) that must be used for its handling).

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:

No, already biosafety level 1. Collagen and FBS have been selected as a biomaterials that best satisfy research objectives.

C1.1.2 Isolation/Segregation

(i) Is/Are the laboratory(s) to be used for this work to be shared with other workers not directly involved in this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, provide details:

Used by researchers

After each culture all shared equipment will be cleaned and decontaminated according to procedures detailed in CBE equipment SOPs. Cultures will be incubated in closed flaks. Risk of cross-contamination will be minimal.

(ii) Is access to the laboratory(s) to be used for this work restricted?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, provide details:

Access to CBE laboratories is restricted to authorised users only. All authorised users have been trained in working in a containment level 2 laboratory; documented training files for all users are available in CBE offices.

Outside of normal working hours the laboratories are locked to ensure safe storage of biological agents and unauthorised entry. Keys are only issued to authorised users who have been granted out of hours access following risk assessment for their intended work.

There is no access to the laboratories by any cleaning or maintenance staff at any time unless a specific permit to work has been granted.

C1.2 Controlling Exposure

C1.2.1 Are sharps (needles, blades, scissors, forceps, glass or capillary tubes) to be used at any stage during this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, list the sharps: Scalpel, glassware, haemocytometer, glass slides/coverslips
If yes, justify there use – is there an alternative? Scalpel required to inflict physical damage to tissue engineered endothelium. There is no alternative. Glass items will be replaced with plastic items where possible. Glass microscope slides and cover slips are essential for microscopy work (SOP033 **use and maintenance of haemocytometer** and SOP022 **use and maintenance of the Olympus CKX41 inverted microscope**
If yes, describe the use and disposal: Disposed of in accordance with CBE COP and SOP003 **disposal of biological (healthcare) waste**
If yes, describe any additional precautions employed to reduce risk: Scalpel will be retained in its protective packaging until required. Following use the scalpel will be deposited immediately in a designated sharps bin

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker i.e. do the work procedures generate aerosols or splashes that pose a risk to workers?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, specify the type(s) and when they will be used: A class II BSC will be used for all cell work in order to reduce the risk of contamination and ensure that any aerosols generated will be contained. BSCs will be operated in accordance with SOP009 **use and maintenance of HERASAFE KS Class II BSC** for cell culture and SOP104 **use and maintenance of HERASAFE KS Class II re-circulating BSCs** for freezing-down using DMSO. A designated biosafety cabinet will be used for scaffold preparation and cell seeding of the scaffolds. Standard aseptic techniques are employed in the handling of cells and scaffolds. For static cultures, the cell suspension is simply added directly to the fabricated scaffolds. To encapsulate cells within the 3D scaffold matrix, the cell suspension is mixed with unpolymerised collagen. Cell-scaffold constructs will be cultured for approximately 2 weeks.	
(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, specify:	

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored?

Cryostorage unit in liquid nitrogen vapour phase. During culture the cells will be maintained in a static CO_2 incubator. Material will be stored according to the following SOPs:

SOP005: Storage and transport of biological materials
SOP008: Receipt of hazardous biological material
SOP013: Use and maintenance if liquid nitrogen stores
SOP079: Use and maintenance of the Heracell CO_2 incubator
SOP031: Cryopreservation and storage of mammalian cell lines

Cell culture medium, FBS and collagen will be stored in the fridge and other reagents such as trypsin will be stored in the freezer (-20°C) according to the following SOPs:

SOP016: Use and maintenance of fridges and freezers
SOP005: Storage and transport of biological material
SOP039: Storage, handling and disposal of chemicals

How will this material be transported within the laboratory e.g. between BSC and incubator? Detail the containment measures which will be used to prevent or contain accidental splashes or spills. Cells will be stored in closed or capped flasks and if transported between or within laboratories will be carried in a second sealed container to prevent accidental splashes or spills. In the event of a spill or breakage SOP038 **Biological spill response** will be followed

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills Any biological material that may need to be transported between labs on site will be done so in accordance with SOP005 Storage and Transport of Biological Agents, and will be sealed within a primary and secondary container

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?						
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)						No
If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):						
Description of material to be shipped (*indicate in available boxes*). Is this:						
Category A		UN2814		UN2900		*Packaging instruction 602 or 620 must be followed*
Or?						
Category B		UN3373		*Packaging instruction 650 must be followed*		
Or?						
Non-hazardous		*Should be packaged to protect sample*				

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly? MCECs are to be purchased from AH Bio. AH Bio is an established supplier of biological materials.

C1.2.7 Centrifugation

(i) If material is to be centrifuged will sealed buckets and rotors be used?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
(ii) Where will these rotors/buckets be opened? Sealed buckets will be opened on the bench top within CL2 laboratories, unless there is a spillage of hazardous material whereby sealed buckets will be opened within a biological safety cabinet.	
(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge Labelled biological spill kits are available in the change area of each laboratory. There are also posters in each lab where there is a centrifuge to provide advice on spillages and reporting procedures. When using a centrifuge, the correct SOP will be followed for the relevant centrifuge and SOP038, "Biological Spill Response" will also be followed.	

C1.2.8 Incubators

If incubators are to be used, what type of incubator (e.g. shaking, static) is used and describe procedures to prevent and contain spillages.

The type of incubator that will be used is a static incubator 5% CO2 at 37°c, SOP079
Spillages will be dealt with according to SOP038 "Biological Spill Response" and specific SOPs for incubators will be adhered to for correct use and maintenance of incubators.

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

1% Virkon is the primary disinfectant and 70% IMS is used for general disinfection and also on surfaces where Virkon cannot be used such as stainless steel surfaces

Have these disinfectants been validated for use with the recipient biological material?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, describe the procedure:

These disinfectants are well known to be effective disinfectants against a wide range of viruses, fungi and bacteria. For Hazard Group1 (or 2), it is sufficient to rely on data from the manufacturer, providing the recommended concentrations and contact times are used. Hence, Virkon (1 %) is used according to the guidelines outlined by the manufacturer and according to standard procedures detailed in the COP and the following SOP006 **Selection and use of Virkon disinfectant**

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored?

A side fastening Howie type lab coat will be worn at all times when working within CL2 laboratories, CBE. These are kept outside the laboratory in the change room.
SOP037 "Use of Personal Protective Equipment" will be followed for guidance on the correct use of PPE.

(ii) What type of gloves will be worn and where will they be stored?

Disposable nitrile powder free gloves for general use will be worn at all times in the laboratory and are stored in designated change rooms/ point of entry into the lab.
Cryogenic gloves will be used when handling samples in liquid nitrogen storage, which are kept in the autoclave room in CBE laboratories.
Heat resistance gloves will used when removing objects from the autoclave, kept in the autoclave room, CBE laboratories.
SOP037 "Use of Personal Protective Equipment" will be followed for guidance on the correct use of PPE.

(iii) Describe any other PPE to be used:

Safety glasses will be worn when advised and face shields will be worn when dealing with the liquid nitrogen stores. Shoe covers are worn at all times within the CL2 laboratories. Safety goggles when using sharps. Correct use of PPE will be used with guidance from SOP037 "Use of Personal Protective Equipment".

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

Hand wash facilities and eye wash stations are available in the change rooms of the CL2 laboratories. Also other hand wash basins are available in analytical laboratories.

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe:	

C1.2.13 Waste Treatment before Disposal

How must waste to be treated before disposal and how has it been validated as being effective?		
Type of Waste	Treatment before disposal	Validation of this treatment
Liquid waste	Virkon decontamination (SOP003 "Disposal of biological waste"	According to manufacturer's instructions; see section C2.1.9
Solid waste	Autoclave decontamination (SOP024 and SOP025)	Treatment cycle validated according to: SOP024 & SOP025, "Use and Maintenance of the Systec VX95 Autoclave"; No CBE044 and No CBE045 in CBE Lab Unit.

C1.2.14 Autoclave sterilisation

If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box			
Type of Waste	Composition of waste	Autoclave cycle (temp, cycle time)	Treatment monitor
Liquid waste	N/R	N/R	N/R
Solid waste	Cell culture consumables (flasks, plates, other plasticware)	Cycle 4 for solid waste, Minimum 121°C for 15 minutes.	Designated autoclave tape monitors
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
CBE – Autoclave room H31	Annual	CBE – In autoclave room H31 (there are two autoclaves)	CBE – In change rooms in yellow bins

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? Yes: After 1% Virkon decontamination for 24hrs, waste is poured down the sink with copious amounts of water in accordance with SOP003 "Disposal of Biological Waste". Liquid waste containing Trypan blue will be dispensed into a Delaware jar which will then be transferred to the Chemical Engineering department for safe disposal.
As solid waste?
Other? N/A

C1.2.16 Solid Waste Disposal

Describe the waste category and disposal route. *(For guidance refer* to http://www.environment-agency.gov.uk)

Colour Code	Categorisation	*Hatch relevant box(es)*	Disposal Method
Yellow	Sharps (not contaminated with cytotoxic/cytostatic material)	X	Yellow Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
Purple/Yellow Special case, contact DSO	Sharps (contaminated with cytotoxic/cytostatic material)	X	Purple/Yellow lidded Sharps bin>clinical waste disposal (incineration @ 1000C)
Yellow	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal)		Yellow rigid one way sealed tissue bins>clinical waste disposal (incineration)
Yellow	Animal body carcasses or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal		Yellow rigid one way sealed tissue bins > clinical waste disposal (incineration)
Special Case – Contact DSO	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preference and is subject to special requirements
Orange	Infected or potentially infected lab wastes that have been pre treated before leaving the site	X	Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > orange clinical waste bags > clinical waste disposal (incineration)
Yellow	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site	X	Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > yellow one way sealed tissue bins > clinical waste disposal (incineration)

C1.2.17 Work with Animals or Vectors (if none proceed to Section C1.2.18)

(i) Are animals or vectors to be infected with any of these biological agents?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	
If yes, describe the procedure and describe where this aspect of the work will be conducted:	
(ii) Is shedding of infectious materials by the infected animals possible or expected?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	
If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:	

(iii) Who will perform the inoculations of animals/vectors? What training have they received?	
Indicate in the adjacent box if Not Relevant (N/R)	
Provide details of the training required:	

C1.2.18 Bioreactor/Fermenters (if none proceed to Section C1.2.19)

Will a bioreactor/fermenter be used to culture a biological agent?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the size, and type of the bioreactor/fermenter.	
(ii) Are any supplementary containment measures required, for example, the use of a BSC or spill tray.	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe:	

C1.2.19 Other Control Measures Required?

No

C1.3 Emergency Procedures

C1.3.1 Describe the procedures in place for dealing with spillages *(specify disinfectants and any special containment for large volumes)*

Within the BSC:

Within the BSC procedures for dealing with small and large spillages are detailed in the following SOPs:

SOP006, "Selection and use of Virkon Disinfectant"
SOP009 "Use and Maintenance of Herasafe KS Class II BSC"
SOP104 "USE AND MAINTENANCE OF HERASAFE KS CLASS II RE-CIRCULATING BIOLOGICAL SAFETY CABINETS".
SOP038, "Biological Spill Response"

Labelled biological spill kits are located within the CL2 laboratories in the CBE laboratories. Posters are placed in the laboratories to enable workers to locate the nearest biological (and chemical) spill kits. Also there are posters near the BSCs displayed in the laboratories to advise on spill response and reporting of spills within the BSC.

Within the laboratory but outside the control measure e.g. BSC, spill tray

For dealing with spillages outside of the BSC but within the laboratory, the procedures are detailed in the following SOPs:

SOP006, "Selection and use of Virkon Disinfectant"
SOP038, "Biological Spill Response"

Labelled biological spill kits are located within the CL2 laboratories in the Wolfson School and CBE laboratories. Posters are placed in the laboratories to enable workers to locate the nearest biological (and chemical) spill kits.

Also there are posters near the BSCs displayed in the laboratories to advise on spill response and reporting of spills within the BSC.

Outside the laboratory e.g. during transport

For transport outside the laboratory, the local code of practice will be followed and also SOP005 "Storage and Transport of Biological Agents" will be followed. In short if biological agents are transported outside the laboratory it will be contained within a primary sealed container which will be sealed within a secondary sealed container. Procedures for dealing with small or large spillages are in place and the following SOPs will be followed:

SOP006, "Selection and use of Virkon Disinfectant"
SOP038, "Biological Spill Response"

Describe the procedures in place for an accidental exposure (if necessary describe different procedures for different types of exposure e.g. eye splash or percutaneous inoculation)

1. Procedures to respond to accidental exposure are detailed in SOP038, "Biological Spill Response" and the local COP. These are detailed in spill response posters located in each laboratory within the CBE CL2 Laboratories. Accident procedures in the case of glass or sharps injury are described in the local COP and displayed in posters located in each CBE Laboratory.
2. Designated hand washing facilities are located in each laboratory change room (and in the Analytical Laboratory (H23) in the CBE Laboratory Unit at Holywell).
3. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room (and in the Analytical Laboratory (H23) in the CBE Laboratory Unit at Holywell).
4. A First Aid Kit is located in the Office outside the Laboratory Unit at Holywell. Signs are posted to enable workers to locate the nearest Medical Kit. Contact details for First Aiders are posted in each laboratory.
5. Essential and Emergency Contact details are posted in each laboratory.
6. Phones are located within designated laboratories within the Laboratory Unit at Holywell.

C2 ASSIGNMENT OF CONTAINMENT LEVEL

The laboratory Containment Level is directly related to each of the 4 Hazard Groups; organisms categorised as HG1 (lowest hazard rating) should normally be handled in CL1 facilities (minimum level of containment), and likewise up to HG4 (highest hazard rating) in CL4 facilities (maximum level of containment). Where the identity or presence of a biological agent is not known the following rules apply: a) where uncertainty exists over the presence of pathogenic biological agent – minimum of CL2; b) where the presence of a pathogenic biological agent is known or suspected – minimum of Containment Level appropriate to the agent, where the assessment is inconclusive but where the activity might involve serious risk – minimum CL3

C2.1. What containment level is required for this work? (see COSHH Schedule 3, Part II for a list of criteria)

Containment level 1 is required for this work, assessed as hazard group 1. However, all procedures will be carried out under containment level 2 (CL2). This is for reasons other than worker protection, including the need to ensure research material is protected and to impose a QA discipline.

C2.2. Describe extra controls or derogation from certain controls

:

N/R

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area

CBE CL2 Laboratory Unit (self-contained laboratory suite and ancillary rooms within the CBE) at Holywell Park	Centre for Biological Engineering	Holywell Park, Loughborough University	Robert Temple (DSO) Chris Hewitt (BGMSA) Carolyn Kavanagh (Lab Manager) Kul Sikand (Lab Manager)

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Davies	OGD	5023010	Research Associate
Liu	YL		Senior Lecturer

C4.2 Information, Instruction and Training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity. Instruction should include the 'Local Rules' or 'Local Codes of Practice' which focus on the working instructions to be followed by all persons involved in the work activity to control or prevent exposure to hazardous biological agent(s). These should be written and readily available to all workers working at Containment Level 2. A formal record of training should be kept for all individuals working at Containment Level 2.

Access to the CBE CL2 laboratories is restricted to authorised users only and personnel are trained according to the local Code of Practice. Prior to authorisation, lab users must complete a training file and obtain the minimum training required by the CBE management and health and safety committee. Individuals involved in the work activity are trained in all procedures and equipment required for the work to be carried out. Training files are ongoing documents that are kept in the CBE office and it is the responsibility of the lab user to identify any further training required to proceed with the project/ begin a new project.

For this project, O.G Davies will partake in practical aspects of this project, whereas Y. Liu will have a more supervisory role

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Davies	1 year MRes training in tissue engineering, 4 years Ph.D training in tissue engineering

C4.4 Other people who may be at risk from the activity e.g. cleaners, maintenance workers or other workers in shared laboratory

Details:

None. Cleaners and maintenance workers are not authorised to enter the laboratory area. All laboratory cleaning is undertaken by authorised personnel. Access for non-laboratory workers is subject to local permit to work procedures. If access is needed for essential maintenance of equipment for example a clean down and decontamination of the laboratories will be performed. This will be documented with decontamination certificates and the maintenance worker fully supervised according to SOP004 "General Laboratory Housekeeping" and the local Code of Practice (COP).

C5 OCCUPATIONAL HEALTH

C5.1 Vaccination

Is an effective vaccination available for any of the pathogens associated with this work? Advice can be obtained from the Occupational Health Adviser (OHA) if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

Hepatitis vaccination series was completed in 2013.

C5.2 Health Surveillance

Is health surveillance required? (Health surveillance is typically applied if working with a hazardous substance that: a) produces an identifiable disease or adverse health effect that can be related to exposure; b) there is a reasonable likelihood that the disease or effect may occur under the conditions of work, and c) there are valid techniques for detecting indications of the disease or effect).

No

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cells, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

C6.1.2 Does This Work Have Ethical Approval? If Yes, Provide Details

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/A

Approval number:			
Date obtained:		Ethics committee name:	

C6.1.3 Are other registrations/notifications required for this work? For example HSE notification under COSHH, Home Office notification under anti-terrorism, crime and security act etc

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, give details:	

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

C7.1.1 Are there any licensing requirements for this work?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. Current procedures to be followed:

- If you wish to import any animal products that you know are not infected with an animal pathogen, or have good reason to expect that they are not infected with an animal pathogen, from within or outside of the EC you must apply for a Research Sample Licence using the Defra form IAPPO1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/iapppo1.htm
- If you wish to import such an animal product but it is known or suspected of being infected with an animal pathogen then you must use DEFRA form IM137. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/inttrade/im137.htm
- If you wish to import an animal pathogen listed under the Specified Animal Pathogens Order then you must use DEFRA form PATH1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/path1.htm

In all cases the instructions for their submission is stated on the forms themselves.

ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

8. DECLARATION

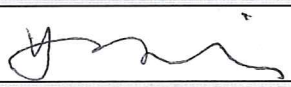
*The declaration must be signed **before** submitting this assessment to the Departmental Safety Officer and University Biological Safety Officer*

I, the undersigned:

- confirm that all information contained in this assessment is correct and up to date
- will ensure that **suitable and sufficient instruction, information and supervision** is provided for all

individuals working on the activity

- will ensure that no work will be carried out until this **assessment has been completed and approved** and that all necessary control measures are in place
- that all information contained in this assessment must remain correct and up to date (the assessment should be **reviewed once a year** and whenever any **significant changes** to the work activity occur)
- will re-submit the assessment for approval if any significant changes occur

Name: Person conducting assessment	**Signature:**	**Date:**
Owen Davies		**10/06/2014**
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Yang Liu		4th July 2014
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**

9.APPROVAL

For work involving **Hazard Group 1** biological agents: Review and approval is required by authorised and designated members of CBE staff before the work begins

For work with **Hazard Group 2** biological agents: Explicit approval is required from the Departmental Biological Safety Advisor and the University Biological Safety Officer before work begins.

If the biological agent has been **Genetically Modified** this form, (approved by the relevant authority, as above) should be submitted with the GMO risk assessment to the Departmental Biological Safety Advisor and both forms forwarded to the LU GM Safety Committee for final approval.

Name: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
A. CHANDRA RA		4 July 2014
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**