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

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/52

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:		Date Approved:	
Version Number:	1	Supersedes *(insert version number if applicable)*	N/A

PART A: Please provide the following general information:

School/Department			
Wolfson School			
Title of Project			
Seeding experiments with Human mesenchymal stem cells			
Project Reference Number:	**J12920**		
Person responsible for this work (*Principle Investigator)*			
Name:	**Andrea Fotticchia**	Position:	**PhD student**
Department:	**Mechanical and manufacturing engineering, Centre of Biomedical Engineering (CBE)**	University School:	**Wolfson School**
Person conducting this assessment			
Name:	**Andrea Fotticchia**	Position:	**PhD student**

Department:	**Mechanical and manufacturing engineering, Centre of Biomedical Engineering (CBE)**	Date Risk Assessment Undertaken:	**23/008/12**
Proposed Project Start Date:	**01/09/12**	Proposed Project End Date:	**15/05/15**

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 purpose is to assess which substrates, selected from an array of electrospun layers, are more suitable to enhance adhesion and proliferation of Human mesenchymal stem cells (HMSCs). The said non hazardous substrates are made of polycaprolactone, a biocompatible polymer.

These experiments will be the first stages of the project which aims to fabricate an artificial biocompatible scaffold for annulus fibrosus regeneration.

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.

1. **Human Mesenchymal Stem Cells (HMSCs) will be seeded onto tissue culture treated plastic (e.g. T175 flasks) and cultured at 37°C, 5% CO_2 until 70% confluent, followed by trypsinisation and subsequent subculture.**
   Cryopreservation and subsequent thawing in subculture will be conducted at various points in the process.
2. **Synthetic electrospun scaffolds, already fabricated, will be used as substrates for cell seeding.**
3. **Cells will be seeded onto the layers and cultured under static conditions.**
4. **The scaffolds will be analysed using stainings and viability assays.**

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. All SOP's available (authorised access only) for review at: https://internal.lboro.ac.uk/restricted/wolfson/CBE_SOP/5_SOPs/SOPs.html.htm.

All work will be carried out in the CBE CL2 Tissue Engineering Laboratory (T208B) located in the Wolfson School, except for cell banking cryostorage procedures, which will be carried out in the CBE CL2 Laboratory Unit located at Holywell Park.

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?
Mesenchymal stem cells	**Bone marrow**	**Human**	**Lonza, UK Primary cell line (existing stock, refer to CBE/BRA/008)**

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

Indicate in the adjacent box if Not Relevant (N/R)		**N/R**
Material type	Species	From where will it be obtained?

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)	**No**
If Yes, provide details of the types of screening and agents screened for:	

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: **Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing cannot offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced cells should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products.**	

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
Human mesenchymal stem cells	**Low risk**	**Well authenticated/characterised cell lines from commercial source. Cells have documented provenance of screening. Cells are categorised as Hazard Group 2 and are to be handled in a containment level CL2 as a precautionary measure.**
		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
HIV-1	**2**
Hepatitis B	**3**
Hepatitis C	**3**

****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: **Cells will be seeded onto T175 flasks or scaffold materials in a Class 2 biological safety cabinet and cultured under 5% CO_2 and 37°C incubator conditions.**	

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 **10^6 cells (40ml media)**	**Per experiment** **~4 x 10^6 (4 Flasks of 40ml each)**

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)	**N/R**
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)	**N/R**
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**
1. Liquid Nitrogen - Cryogenic processing. **2. Trypan Blue - Carcinogenic agent used for cell Counting.** **3. Dimethyl Sulfoxide (DMSO) – Cytotoxic agent used for freezing down working cell bank.** **4. Formalin – Toxic agent used to fix scaffolds prior to histology.**	

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

1. Use of cryogenic stores will be carried out only by an authorised user in accordance to SOP013 and use of appropriate PPE.
2. Use of Trypan blue used according to COSHH form CBE32.
3. DMSO - if cells are to be used to prepare a working cell bank, procedure will be carried out according to COSHH form CBE41 and SOP031 ("CRYOPRESERVATION AND STORAGE OF MAMMALIAN CELL LINES").
4. Formalin used according to COSHH form 69.

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
Foetal bovine serum (FBS)	**Unknown**	**Bovine foetus**	**Foetus**	**Commercial supplier Lonza, UK. Sourced from Brazil according to Material Safety Data Sheet.**

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 **NOTE: FBS product contains material of animal origin. The material contains no hazardous or toxic substances. The material is supplied by a commercial company and sent through the post in secure packaging. Material is liquid and stored frozen at -20C.**	

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
FBS	**Product is certificated Likelihood that it contains substances hazardous to health is low. Refer to certificate of analysis.**	**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
1. Not categorised (refer to certificate of analysis)	**None. Animal proteins may be a potential contact irritant**	**Well authenticated/characterised product from commercial source.**
		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

B4.4.2 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:

To achieve the objectives of the project as it had been planned, No suitable replacement exists.

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:

Access to all CBE Containment level 2 labs is restricted to authorised workers with appropriate training in accordance with documented local Code of Practice and Quality Management System requirements.
All work will be carried out in the CBE Tissue Engineering Lab, T208B located in the Wolfson School-except for Nikon Eclipse Ti optical microscope (SOP072) which is located in Holywell park CBE lab.
The BSC, incubator and pipettes will be shared with Husnah Hussein, a PhD student, the supervisor and undergraduate students to whom the supervisor gave the authorization. It is not expected that anybody else except the HMSCs user will get in touch with the cells or the consumables used for their handling.

Access to all CBE CL2 laboratories is not permitted for any cleaning or maintenance staff at any time unless a specific permit to work has been granted.

(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 is restricted to people with documented training (authorised access documented in the training record of each individual) in accordance with the COP and QMS.

The T208 laboratory is locked at all times on exit to ensure safe storage of biological agents and unauthorised entry. The CBE T208B Laboratory is locked at all times outside of normal working hours Keys to the laboratory are only issued to authorised users. Out of Hours/Lone working is logged and permitted subject to risk assessment.

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:

1) Disposable plastic forceps
2) Plastic pipette tips
3) Haemocytometer glass slide
4) Disposable surgical blades
5) Metal forceps

If yes, justify there use – is there an alternative?

It is local practice in the laboratory unit that the use of sharps is avoided wherever possible. Glass items are replaced with plastic alternatives where possible (as (1) and (2)).

The haemocytometer is necessary to perform the manual cell count; the glass slide is an essential component, according to SOP033 "USE AND MAINTENANCE OF HAEMOCYTOMETER".

The substrate upon which cells are cultured need to be cut in the most accurate way to avoid that scaffold are damaged by improper handling. To achieve the same quality level no suitable alternative is available.

To finely handle the sample plastic forceps with blunt end are not sufficient.

If yes, describe there use and disposal: **1) Sample handling** **2) Growth medium replacement** **Used sharps are placed directly into approved sharps containers (for non-cytotoxic waste). Sharps bins are removed when three quarters full and contents rendered safe by autoclaving prior to their removal from site.** **3) The haemocytometer glass slide, as non disposable device, will be washed with water and ethanol 70% according to the SOP033 "USE AND MAINTENANCE OF HAEMOCYTOMETER"** **4) Sample cutting** **Used blades are placed directly into approved sharps containers (for non-cytotoxic waste). Sharps bins are removed when three quarters full and contents rendered safe by autoclaving prior to their removal from site.** **5) Sample handling** **Forceps are washed in ethanol 70% after each usage. Prior to be used the following time they are sterilized in autoclave so that any biological contaminant is eliminated.**
If yes, describe any additional precautions employed to reduce risk: **Current precaution sufficient. Accident procedures for sharps and glass injuries are displayed in posters in all labs within the CBE CL2 laboratories**

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: **Class II Biological Safety Cabinet will be used for all manipulations that may produce aerosols or splashes of the biological material, according to the following SOPs** **1) SOP104, "Use and Maintenance of HERASAFE KS Class II re-circulating BSCs"** **2) SOP105, "Use and Maintenance of the Faster Class II BSC"** **Using HG1 and HG2 materials, this control measure is primarily to ensure protection of research materials (as part of a local quality assurance discipline)**	
(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? ***Cell line listed in B2.1.1 are stored in freezer (-80˚C) prior to usage. After defrosting they will be handled in the BSC and, at the end, transferred to an incubator.*** ***Medium is stored in the fridge, trypsin in the freezer (-20˚C)*** ***According to the following SOPs :*** ***1) SOP005, "Storage and Transport of Biological Materials"*** ***2) SOP114, "Use and Maintenance of the HERAcell 150i CO2 Incubator"***
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. ***Seeded samples are placed in multiwell, which will be covered with designated lid. All other transfers within T208B Laboratory in closed secondary containers large enough to carry the designated material.*** ***Appropriate spill response procedures are posted in the lab and documented in detail in the following SOPs:*** ***1) SOP005, "Storage and Transport of Biological Material"*** ***2) SOP038, "Biological Spill Response"***

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 ***No transport anticipated outside the T208B laboratory. However, any transport will be subject to controlled procedures according to the local COP and SOP005 (see below). For example, if necessary, transfers will use double containment procedures – sealed primary containers inside sealed secondary containers. Potentially hazardous waste is autoclaved in situ (T208B Lab and CBE Lab Unit).*** ***1) SOP003, "Disposal of Biological Waste"*** ***2) SOP005, "Storage and Transport of Biological Material"*** ***3) SOP038, "Biological Spill Response"***

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?

Existing stock of cellular materials located in the Wolfson T208B lab.

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 within the Containment Level 2 (CL2) Labs, unless there is evidence of a potential spillage, in which case the sealed buckets will be opened in the BSC (SOP114, "Use and Maintenance of the HERAcell 150i CO_2 Incubator").

The centrifuge is operated and maintained according to the following SOPs:

1) **SOP128, "Use and Maintenance of Heraeus Centrifuge Biofuge Primo R"**
2) **SOP038, "Biological Spill Response"**

(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge

Procedures to prevent, contain and respond to leakages and spillages in the centrifuge are detailed in the following SOPs:

1) ***SOP128, "Use and Maintenance of*** **Heraeus** ***Centrifuge Biofuge Primo R"***
2) ***SOP038, "Biological Spill Response***

Glass breakage cannot occur because plastic vials are going to be used.

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.

Static incubators are used. Procedures to prevent, contain and respond to spillages in the incubators are detailed in the following SOPs:

1) ***SOP114, "Use and Maintenance of the HERAcell 150i CO2 Incubator"***
2) ***SOP038, "Biological Spill Response".***

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

The disinfectants were carefully chosen for effectiveness of use. The number of disinfectants in use is strictly limited to avoid errors and ambiguities in use and accidental mixing of compounds that may give rise to hazardous reactions or the formation of toxic products. Unless there are compelling reasons to do so otherwise, Virkon (1 % w/v) is the sole disinfectant used in the laboratory other than 70 % IMS which is used for general disinfection cleaning (SOP004) where Virkon cannot be used (e.g. stainless steel surfaces).

Virkon has a wide range of bactericidal virucidal, fungicidal activities. Representative viruses from all the major virus families are inactivated by Virkon. Working solutions of 1 % w/v have low toxicity and no

irritancy. Selection and procedures are detailed in the following SOPs: **1) SOP004, "General Laboratory Housekeeping"** **2) SOP006, "Selection and Use of Virkon Disinfectant"** **3) SOP039, "Storage, Handling and Disposal of Chemicals"** **COSHH Risk Assessment reference for Virkon CBE/039 will be reviewed prior to use.**

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:

They 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 SOP:

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?

Side fastening Howie type lab coats are worn. They are stored outside the laboratory in designated change room Proper use of PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)".

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

1) **Autoclave gloves.**
2) **Latex powder free gloves for general use, which will be stored in the change rooms and/or point of entry to the CBE laboratories.**

Correct use of PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)"

(iii) Describe any other PPE to be used:

1) ***Laboratory safety glasses (including those for spectacle wearers) when handling dusty materials on an open bench.***
2) ***Face Shields (primarily for handling liquid nitrogen).***
3) ***Shoe covers mandatory in the CBE at Holywell and in case of a spillage in the CBE at Wolfson.***
4) ***Aprons or disposable lab coats for extra protection over Howie type laboratory coat.***

Correct use of the above PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)". Goggles stored in the lab

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

1) Designated hand washing facilities are located in laboratory change room.
2) Eye Wash stations are located next to 'hand washing sink only' in the laboratory change room

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 sterilisation (SOP003 – Disposal of biological waste)***	***According to manufacturer's instructions; see section C2.1.9***
Solid waste	**Autoclave sterilization (SOP003 – disposal and disinfection of biological waste)**	***Treatment cycle validated according to (1) SOP054 in the T208B Lab (1) SOP024 & SOP025, "Use and Maintenance of the Systec VX95 Autoclave"; No CBE044 and No CBE045 in CBE Lab Unit (back-up)***

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/A	N/A	N/A
Solid waste	**Multiwells, forceps, tips, vials**	**Cycle 4 for solid waste using SOP054 (CBE lab Unit T208B).**	**Designated autoclave tape monitors**
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
Autoclave located in the T208B Lab Unit at Wolfson.	**UKAS calibration on 20th February 2012 for cycle 4**	**Autoclave CBE-045 and CBE-044 in Autoclave Room (H31) in the CBE Lab Unit at Holywell.**	**In designated area within the T208B Tissue Engineering Lab [or transferred to secure cage within the CBE Lab Unit at Holywell]**

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? **Yes: Disinfected with Virkon and disposed of to drain with copious amounts of water in accordance with SOP003 – " Disposal of Healthcare waste"**
As solid waste? N/A
Other?

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)		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)		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		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		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)	No
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)	No

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) | 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) | No
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) | No
If yes, describe:

C1.2.19 Other Control Measures Required?

None

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: **Procedures for dealing with small and large spillages are detailed in the following SOPs:** **1) SOP006, "Selection and use of Virkon Disinfectant"** **2) SOP104 "USE AND MAINTENANCE OF HERASAFE KS CLASS II RE-CIRCULATING BIOLOGICAL SAFETY CABINETS".** **3) SOP038, "Biological Spill Response"** **Labelled Biological Spill kits are located in each laboratory within the CBE Lab Unit and within the Tissue Engineering Laboratory (T208B). Signs are posted to enable workers to locate to the nearest biological (and chemical) spill kits. Posters are also displayed where a BSC is located to advise on spill (inside the BSC) response and reporting procedures. No large volumes of biological material are used.**
Within the laboratory but outside the control measure e.g. BSC, spill tray

Procedures for dealing with small and large spillages are detailed in the following SOPs:

1) **SOP006, "Selection and use of Virkon Disinfectant"**
2) **SOP038, "Biological Spill Response"**

Labelled Biological Spill kits are located in each laboratory within the CBE Lab Unit and the Tissue Engineering Laboratory (T208B). Signs are posted to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed to advise on spill (outside the BSC) response and reporting procedures.

Outside the laboratory e.g. during transport

No transport outside the lab is anticipated except for waste that is made safe before leaving the lab

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 the laboratory. Accidental procedures in the case of glass sharps injury are described in the local COP and displayed in posters located in the laboratory.
2) Designated hand washing facilities are located in the laboratory change room.
3) Eye Wash stations are located next to the 'hand washing only' sink in the laboratory change room.
4) A First Aid Kit is located in the change room of the T208B Lab. Signs are posted to enable workers to locate the nearest Medical Kit. Contact details for First Aiders are posted in the laboratory.
5) Essential and Emergency contact details are posted in the laboratory.

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)

All procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Tissue Engineering Laboratory Unit. This project, involving the use of Hazard Group 2 Biological Agents will be carried out at Containment Level 2 that ensures research material protection (e.g. the use of a class II safety cabinet) and to impose a quality assurance discipline.

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

None

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area

1.CBE Tissue Engineering Laboratory Unit (T208B) **2. CBE Laboratory Unit (*self contained laboratory suite and ancillary rooms within the CBE*)**	**1.Wolfson School of Mechanical and Manufacturing Engineering** **2.Holywell Park**	**Loughborough University**	**Carolyn Kavanagh** **Kulvindar Sikand** **Yang Liu** **Bob Temple**

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Fotticchia	**AF**	**B130783**	**PhD student**
Liu	**YL**	**5003393**	**Supervisor**

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.

Identified personnel are trained in required procedures and equipment and instructed against local Code of Practice and QMS. Formal records of training are kept for all workers authorised to work at Containment Level 2 (CL2) within the CBE CL2 Laboratory Units.

Yang Liu is the main supervisor for the project and will be acting in a supervisory role. All practical work carried out by Andrea Fotticchia is subject to conditions identified and recorded in the training file.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Fotticchia	**Documented in Personal Training File**

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. All laboratory cleaning is undertaken by authorised personnel (i.e., CBE staff). Access for non-laboratory workers is subject to a local permit-to-work procedure. If access is needed for essential maintenance of equipment (e.g. a clean down) a decontamination of the laboratories will be performed. This will be documented with decontamination certificates and the maintenance worker will be fully supervised according to SOP004 "General Laboratory Housekeeping" and the local Code of Practice.

Two laboratory shut downs occur every year for ~week for maintenance work to be done in the CBE Laboratories. Prior to these shut down weeks, a full deep clean decontamination will be performed in all laboratory areas.

All other workers in the CBE Laboratories are authorised personnel.

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

Yes - Hepatitis B vaccination. Recorded in Training Record.

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)			No
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:**
Andrea Fotticchia	Andrea Fotticchia	24/8/12

Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
Yang Liu		11th Sep. 2012

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**
Paul Hourd (CBE QM)		
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**
C. M. MOORE	C. M. Moore	12/9/12

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CERTIFICATE OF ANALYSIS

Product Code: DE14-801F
Product: FBS - Brazilian Origin 500ml

Lot Number: 0SB016
Manufacture Date: 02-Sep-2010
Expiration Date: 02-Sep-2015

TEST (Method)	SPECIFICATIONS Min.	Max.	Results
Sterility (EP and USP)	Not Detected	Not Detected	Not detected
Mycoplasma detection E.P.	Not Detected	Not Detected	Not detected
pH @ 20-25°C (EP)	6.8	8.3	7.1
Osmolality (mOsm/kg)	280	365	319
Hemoglobine, mg/100ml	***	</= 30	13.0
Total Protein, g/100ml	3	6	4.2
IgG Concentration, mg/ml	***	</= 0.5	0.077
Endotoxin	***	</= 50	1.630 EU/ml
Cell Growth assay	Pass	Pass	Pass
Virus Testing by Cell Culture			
BVD	Not Detected	Not Detected	Not detected
IBR	Not Detected	Not Detected	Not detected
PI-3	Not Detected	Not Detected	Not detected

The country of origin of the raw material used to manufacture the above referenced product is Brazil. Brazil is free of Bovine Spongiform Encephalopathy in accordance with the Organization of International Epizooties (OIE). A geographical BSE risk assessment report (GBR) by the European Food Safety Authority has been issued in June 2005 and designated Brazil as a GBR level II risk (unlikely but can not be excluded that domestic cattle are infected with the BSE agent).

This lot has been reviewed by Quality Assurance in compliance with requirements of Lonza's Quality System.
This document was generated from a validated Part 11-compliant electronic system and thus handwritten signatures are not required.