Loughborough University **Biological Risk Assessment**	Safety Department use only		Material(s) Classification	
	Reference Number:		Hazard Group 1	☑
			Hazard Group 2	☐
	CBE Use only		GMO	☐
	Reference Number:	CBE BRA 185	HTA Licensable	☐

FORM CBE-RA-Form/002 Version 1.0

RISK ASSESSMENT AND PROJECT REGISTRATION FOR WORK INVOLVING BIOLOGICAL MATERIAL

PLEASE READ CAREFULLY

This form acts to register projects involving the use of Biological Agents and / or Genetically Modified Micro-Organisms, or of materials that may be contaminated with these agents. It assesses the hazards and risks associated with the project as well as identifying those at risk and the measures necessary for preventing, or controlling these risks. Please ensure that sufficient detail is provided when completing this form and that the relevant written SOPs are referenced where required. Once completed and approved, all risk assessments must be supplied to all those working within this project. The work described within this form must not commence until this risk assessment has been completed and approved and that all necessary control measures are in place.

Any changes to the work, or the persons involved, must be notified to the authorised person. All changes requested must be recorded within the risk assessment change control form and may also need to be incorporated within an amended version of this form.

A separate risk assessment will be required for assessing risks associated with GMO activities.

The following declaration must be completed and undersigned by the Principal Investigator or Person Responsible for the project

- All information contained in this form is accurate and comprehensive.
- All workers involved will be instructed that their work must remain within the boundaries of this project registration & assessment.
- All workers have been given, or will be given before they become involved, adequate training and where necessary their competency assessed.
- All workers have, or will be before their involvement begins, enrolled with Occupational Health for health clearance where necessary.
- It is understood that this risk assessment shall not be transferred to a third party without the PI/Supervisor/Line Manager named in this form either taking responsibility for the new activities, or ensuring that a new proposal is submitted.
- All changes to the work covered by this form will be reassessed & the changes submitted to the authorised person before those changes are made to the work.

Principal Investigator		Person conducting this risk assessment	
Name	Karen Coopman	Name	Lisa Barrett
Position	Reader in Biological Engineering	Position	Research Assistant
Department	Chemical Engineering	Department	Chemical Engineering
School	AACME	School	AACME

The Project Activity			
Title	Using Growdex to encapsulate cells		
Reference Number			
Start Date	13 Nov 2019	End Date	30 Sep 2020

Others involved in the work	
Names	

Name	Lisa Barrett	Signature	Lisa Barrett	Digitally signed by Lisa Barrett Date: 2019.11.13 14:13:37 Z	Date	13 Nov 2019

1. INTRODUCTION

1.1 Background & aim of project	1.1 Mesenchymal stem cells can be stored in Growdex, a biocompatible hydrogel, at ambient and refrigerated temperatures, however yield of recovered cells is low. This project aims to improve recovery of cells by reducing the size of the gel to microparticles and encapsulating cells within these. Cell recovery from GrowDex will be performed using an enzymatic digestion of GrowDex and cell viability assays will be used to determine viability and yield. The project also aims to improve cell yield by testing different medium formulations and additives to support cell survival at ambient and refrigerated temperatures.
1.2 Description of experimental procedures	1.2 Passaging cells - Cells will be looked at under a microscope and passaged at 70-80% confluency. Culture medium will be aspirated from cells in a T flask, cells will be washed with PBS and trypsin/EDTA will be added with incubation for 5 minutes to detach cells from flask. DMEM medium supplemented with ultraglutamine and FBS will be added and the resulting cell suspension will be centrifuged at 200 g for 5 minutes. A 200 ul sample of the cell suspension will be used for cell counting using an NC-100 Nucleocounter. The cell pellet will be resuspended in fresh culture medium and seeded into a new flask. Flasks will be incubated at 37C with 5% CO2. Feeding cells - Medium will be aspirated from flasks and replaced with fresh, warmed culture medium and returned to the incubator. Encapsulation of cells in GrowDex - GrowDex/GrowDex-T dilutions will be made by adding culture medium to GrowDex/ GrowDex-T and stirring and pipetting to mix. Cell suspensions will be prepared as above and added to the GrowDex/ GrowDex-T dilutions by stirring and pipetting. The resulting cell/GrowDex mixture will be pipetted into 24-well low adhesion plates and incubated either at 37C with 5% CO2, at ambient temperature or at 4C in a refrigerator. Cell viability assays - Cells encapsulated within GrowDex/GrowDex-T will be monitored using a PrestoBlue cell viability assay or a live/dead fluorescence-based viability assay. For the PrestoBlue assay, the PrestoBlue reagent will be added directly to the well plate and incubated at 37C for 1 - 4 hours. Absorbance will be measured using a plate reader. For the live/dead assay, medium will be aspirated from cells, staining solution will be added and the cells will be incubated for 30 minutes at room temperature. Cells will then be imaged using a fluorescence microscope. Release of cells - GrowDase enzyme stock solution will be diluted with culture medium and added to cells encapsulated within GrowDex/GrowDex-T. Plates will be incubated at 37C for up to 12 hours, with or without a plate shaker. Viability of released cells will be determined using an NC-100 Nucelocounter. Differentiation assays - The capacity of released cells to differentiate will be determined by differentiation assays according the the manufacturer's protocol at a later date. Encapsulation of cells within GrowDex microparticles - This will be carried out at a later date and is covered by the process risk assessment 'Membrane emulsification to encapsulate mesenchymal stem cells'.
1.3 Where will this work be carried out?	Rooms/areas: H23, H34
	Building(s): Centre for Biological Engineering, Garendon Wing, Holywell Park

☑ **2.1 Human or animal tissues, cells, body fluids or excreta will be used in this project**

2. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

2.2 List all cells, tissues, body fluids and excreta to be used. For cells, indicate primary, continuous or finite.

Material type	Organ source	Species	Where it will be obtained from (Include country of origin)
Mesenchymal stem cells	Bone marrow	Human	Orignally from Lonza (USA)- will be using previously banked down cells from the CBE cryostores- no longer HTA relevant

☐ **2.3 Material(s) listed in section 2.2 above are considered to be 'relevant material' under the Human Tissue Act 2004.**

☐ **2.11 Biological agents will be used in this project**

3. CLASSIFICATION OF HAZARD GROUP

3.1. Are you confident that any non-GM organism, tissue, cell, body fluid, excreta or any component thereof covered by this assessment cannot potentially pose a threat to humans or cause human diseases?	⊘ Yes - Classify as HG1
3.1.1. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause human disease and potentially be a hazard to humans but is unlikely to spread to the community and for which there is usually effective prophylaxis or treatment available?	○ Yes - Classify as HG2
3.1.2. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause severe human disease and potentially be a serious hazard to humans and that may spread to the community, where effective prophylaxis or treatment may or may not be available?	○ Yes

3. CLASSIFICATION OF HAZARD GROUP

3.2. Do any of the materials contain pathogens or toxins covered by the Anti-Terrorism Crime and Security Act?	○ Yes	ATCSA Schedule 5
ASSIGNMENT OF CONTAINMENT LEVEL	CL2	

4. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

4.2. Will any culturing of the material described in section 2 take place? *If Yes, describe which cell(s) will be cultured and under what conditions.*	☑ Yes ○ No	Cells will be cultured initially under standard conditions of 5% CO2 and 37C. Cells will also be incubated at ambient (room) temperature and at 4C in the fridge.
4.3. Could HIV permissive cells be present*? *If Yes, describe the cells and for how long these cultures will be allowed to grow.* *If unsure seek advice. Refer to CBE Code of Practice for details on additional precautions.*	○ Yes ☑ No	
4.4. What is the maximum volume of culture grown?	Per Vessel	30
	Number of vessels	10
4.5. Will the tissues, cells, body fluids or excreta be manipulated in any way that could result in the concentration of adventitious biological agent present? *If Yes, explain.*	○ Yes ☑ No	
4.6. Will any of the tissues, cells or fluids be donated by you or your colleagues working in or with access to the labs?	○ Yes ☑ No	

5. RISKS AND CONTROL MEASURES

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.1. Might infectious droplets, aerosols or splashes be created, either deliberately or by accident?	☑ Yes ○ No	Cell culture will be carried out in Class II BSCs using aseptic technique. If spills occur, the spill procedure as outlined in the SOP will be followed. Briefly, small spills will be covered with 1% virkon soaked paper towels for 10 minutes. Large spills will be cordoned off and left for 30 minutes for aerosol to dissipate, a clean up team will be assembled and virkon powder will be used to soak up the spill. PPE will be worn at all times while working in the labs.	SOP037 SOP038
5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☑ Yes ○ No	Flasks and plates will be transported between the BSC, incubator, fridge and benchtop using due care and diligence. This will include making sure that lids are properly closed to prevent spillages and also infection of cells. Making sure that there are no trip hazards present in the lab before work begins.	
5.3. Will this material (including waste) be transported locally between sites on campus but outside the laboratory?	○ Yes ☑ No		
5.4. Will material(s) listed in section 2.2 or section 2.3 be shipped to organisations elsewhere in the UK or abroad?	○ Yes ☑ No		
5.5. Will this material be received from organisations elsewhere in the UK or abroad?	☑ Yes ○ No	The MSCs were originally purchased from Lonza. These have been banked down and are currently stored within the CBE cryobanks. The cells arrived as Bone marrow aspirate, but have been grown on cell culture plastic, so no original material is left, before they were banked down. They are no longer HTA-relevant.	SOP008

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.6. Will this material be stored?	☑ Yes ○ No	Cells will be stored in the vapour phase of liquid Nitrogen. Correct PPE will be worn when transferring cells to and from liquid nitrogen as per the SOPs. When in culture, the cells will be stored in incubators at 37C and 5% CO2, at 4C in the fridge or on the benchtop at ambient temperature.	SOP031 SOP013
5.7. Will infectious material be centrifuged?	☑ Yes ○ No	The cells will be centrifuged in either 15ml or 50ml centrifuge tubes. The centrifuges will be balanced with equal volume balances before being started. The centrifuges will be set to 200xg for 5mins. They centrifuge tubes will be checked to make sure they are closed before being placed into the centrifuges and lids will be secured over the buckets before operating the centrifuge. Spillages inside the centrifuge will be dealt with as per the SOP.	SOP047 SOP038
5.8. Are biological samples to be cultured in an incubator?	☑ Yes ○ No	Cells will be cultured in an incubator under standard conditions. Temperature and CO2 levels will be regularly checked and any irregularities will be reported to the lab manager. Incubators used as per the SOP114. If spills occur they will be cleaned up with 70% IMS, or if large as per the SOP038.	SOP114 SOP038
5.9. Are sharps to be used at any stage during this activity?	☑ Yes ○ No	Pipette tips will be used during cell culture and GrowDex preparation. These will be placed in the yellow autoclavable sharps containers for safe disposal. Sharps containers will not be filled more than three quarters full. If a sharp injury occurs, the wound will be washed immediately and the lab manager, first aider and safety officer are informed. A near miss/accident form also needs to be completed.	SOP003
5.10. Are animals to be used in this project?	○ Yes ☑ No		
5.11. Will a fermenter / bioreactor be used to culture a biological agent or material?	○ Yes ☑ No		
5.12. Is there any stage within the experimental procedures when an infectious material is inactivated (other than for disposal)?	○ Yes ☑ No		
5.13 Are any of the following to be used in conjunction with the project?	☐ Carcinogens or Mutagens		
	☐ Toxins		
You must complete a cryogen risk assessment before work begins and add the reference here.	☑ Liquid Nitrogen	Used for storage of cells.	SOP013
	☐ Ionising radiation		
	☐ Lone working		
5.14. Are there any conditions associated with the hazards described in section 5.13 that require additional control measures?	○ Yes ☑ No		

6. PPE AND HYGENE

Control Measure	Details		Reference to SOPs / other documentation
6.1 When will gloves be worn?	At all times in the lab		SOP037
6.2 What type and where will they be stored?	Nitrile for general lab work. Insulated gauntlet gloves for dealing with liquid nitrogen. Heat resistant gloves for operating the autoclaves.	In Lab and in Changing Area	SOP037 SOP013 SOP031
6.3 When will laboratory coats be worn and what type are these?	At all times in lab	White Howie	SOP037

Control Measure	Details		Reference to SOPs / other documentation
6.4 Where will lab coats be stored and what are the arrangements for cleaning or disposal?	Stored in first change	Lab coats are cleaned once a month, or if they come into cc	SOP037
6.5 Provide details of any other types of PPE to be used?	Shoe covers are to be worn at all times over closed toe shoes. No bare legs. Safety glasses to be worn when operating the autoclave, cleaning the aspiration line and working with hazardous material. Face visor to be worn when dealing with liquid nitrogen.		SOP037
6.6 Describe the lab hygiene facilities available and where they are located	Sinks and eye wash stations	n change areas and labs	SOP037
6.7 Where are the first aid boxes and emergency spill kits located?	First aid kit - Office and First	Biological spill kits - all change rooms and H31	

7. WASTE

7.1 How will waste be treated prior to disposal

(Note that all differently treated wastes must be included e.g. if some liquid is autoclaved, but others not, then describe both)	Treatment prior to disposal	Is the treatment validated?	Reference to SOPs / other documentation
☑ Liquid waste	Liquid waste can be autoclaved or treated with Virkon for 24 hours then discarded down the drain with copious amounts of water	☑ Yes ○ No	SOP003 SOP025
☑ Solid waste	Solid waste contaminated with biological agents which does not contain chemicals can be autoclaved on cycle 4, and discarded via the orange waste stream. If solid waste contains and disinfectant or chemicals, this must be disposed of via the yellow waste stream.	☑ Yes ○ No	SOP003 SOP025
☐ Other (Specify)			
7.2 Is any waste being autoclaved?		☑ Yes ○ No	SOP003 SOP025
All cycles have been validated for the actual load types used? *(If Yes, documentary evidence of the validation must be available)*		☑ Yes ○ No	SOP025
The successful completion of every load is checked prior to disposal?		☑ Yes ○ No	SOP025

7.3 How will liquid waste be disposed of?

☑ To drain?	Autoclaved or virkon treated liquid waste can be poured d	☑ Yes ○ No	SOP003 SOP025
☐ As solid waste?			
☐ Other (Specify)			

7.4 How will solid waste be disposed of?

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☑ Sharps	**Orange**	Yellow/Orange lidded sharps bin > autoclave sterilisation if known or potentially infected > clinical waste disposal (incineration)
☐ Sharps contaminated with cytotoxic or cytostatic material		

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☐ Human body parts, organs, including blood bags and blood preserves and excreta that have been pretreated before leaving the site		
☐ Animal body carcasses or recognisable parts that have been pretreated before leaving the site		
☐ Potentially or known infected lab wastes contaminated or potentially contaminated with cytotoxic or cytostatic material that have **NOT** been pretreated before leaving the site		
☑ Potentially or known infected lab wastes that have **NOT** been pretreated before leaving the site	**Yellow**	Yellow clinical waste bags > clinical waste disposal (incineration)
☑ Infected or potentially infected lab wastes that **HAVE** been pretreated before leaving site	**Orange**	Disinfection or sterilisation in the lab site > orange clinical waste bags > clinical waste disposal (incineration)

8. MAINTENANCE

8.1 Are preventative maintenance and monitoring regimes in place for the following laboratory equipment?

	Inspection / Servicing Frequency	Cleaning / Disinfection Frequency	Monitoring / Alarms Frequency	Reference to SOPs
☑ Centrifuges	User inspection before each use. Weekly checks. Serviced after 100-150 hours use.	Weekly	With each use	SOP047
☑ BSCs	Weekly	Before and after use, also weekly clean	Before each use - downflow velocity and performance factor are checked and recorded	SOP009
☐ Fume Hoods				
☑ Autoclaves	Inspected before each use	Monthly	Before each use	SOP025
☑ Incubators	Weekly	Fornightly/monthly/ when required	With each use	SOP114
☑ Liquid N_2 Stores	Biweekly	Biweekly	Constant	SOP013
Failure contingency plan	There is a spare liquid nitrogen store which is at temperature, where the cells can be moved into if there is a failure with one of the liquid nitrogen stores.			
☑ Freezers	Monthly - inspection, maintenance and temperature check	Biannually	Constant	SOP016
Failure contingency plan	There is a spare freezer within the CBE which is at temperature, can be used if one of the freezers fails by transfering the contents between the two.			
☑ Fridges	Biannually	Biannually	Constant	SOP016
Failure contingency plan	There is a fridge within the CBE which is at temperature which can be used if another fridge fails.			
☐ Others				

9. TRAINING

9.1. Have all project research workers undertaken safety training for working with hazardous or potentially hazardous biological materials and agents at CL2?

☐ 9.2. This work involves HTA 'Relevant Material', confirm that all project research workers have undertaken HTA training

Name of researcher	Had Training	Date training completed (or will be completed)	If no, state why
Lisa Barrett	(•) Yes ( ) No	Oct 2019	

☐ 9.2. This work involves HTA 'Relevant Material', confirm that all project research workers have undertaken HTA training

10. EMERGENCY PROCEDURES

10.1 Are procedures in place for dealing with spillage of infectious or potentially infectious material

Equipment	Reference to SOPs
☑ Within the BSC	SOP038
☑ Within the centrifuge	SOP038
☑ Within the laboratory, but outside any primary control measures (e.g. BSC)	SOP038
☑ Outside the laboratory	SOP038

Are procedures in place for the security of these HTA Relevant samples?

☐ Loss or theft of samples (including whilst in transit)	
☐ Loss of traceability of samples	
☐ Incorrect disposal of samples	

10.2 Describe the procedures in place for an accidental exposure

Immediate action	Skin exposure-flush with running water and wash with soap. Eyes-flush with eyewash for 15 minutes Sharps injury-encourage bleeding and seek medical attention.	Ref to SOP's	SOP038
When and whom to report the incident	Contact first aider and report to lab manager and DSO. Complete the	Ref to SOPs	SOP038

11. ACCESS

		Explanation	References
11. Is/are the lab(s) adequately separated from other areas (e.g. offices)?	☑ Yes ( ) No		
11.2. Is/are the lab(s) or other work areas shared with other users not involved in the project?	☑ Yes ( ) No	Work areas will be shared with users working on other projects. Other lab users will be informed of the type of work being carried out and alerted to any potential hazards. Work will be carried out in BSCs and any work areas cleaned before and after use.	SOP004
11.3. Describe the measures in place to ensure that hazardous biological agents or **HTA relevant** material is secure	☑ Yes ( ) No	Labs can only be accessed by other authorised users who have undergone safety training. All authorised lab users are made aware that HTA material is is use in the lab by some users and are instructed not to move it.	

12. OCCUPATIONAL

12. OCCUPATIONAL	
12.1. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunisation. Have all workers involved in this project been immunized?	☑ Yes ○ No
12.2. Is health surveillance required?	○ Yes ☑ No

13. NOTIFICATIONS	
☐ 13.1. Are any of the cells, tissues or fluids covered by the Human Tissue Act (HTA) under the University HTA Licence?	
☐ 13.2. Are any of the cells, tissues or fluids obtained from a HTA licensed biobank with REC approval for generic research use?	
☐ 13.3. Does this work have ethical approval from a recognised NHS Research Ethics Committee?	
☐ 13.4. Does any of the work require approval from the University Ethical Committee?	
☐ 13.5. Do any of the materials require approval for use from the UK Stem Cell Bank Steering Committee (MRC)?	
☐ 13.6. Do any of the materials or biological agents listed require any other licenses?	

14. APPROVALS	
Authorised Person	Karen Coopman Digitally signed by Karen Coopman Date: 2019.11.14 09:49:30 Z 6/12/19
Departmental Biological Safety Advisor	