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 171	HTA Licensable ☐

FORM CBE-RA-Form/002 Version 0.3

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	Dr Elizabeth Ratcliffe	Name	Bridie Catchpole
Position	Vice-Chancellor's Lecturer in Biological Engineering	Position	PhD student
Department	Centre of Biological Engineering	Department	Chemical Engineering
School	AACME	School	AACME

The Project Activity			
Title	Biocompatibility of PCL-PGS-Bioglass scaffolds for cardiac patch applications		
Reference Number			
Start Date	10/12/2018	End Date	01/06/2019

Others involved in the work	
Names	Dr Jamie Christie
	Dr Elisa Mele

Name	Elizabeth Ratcliffe	Signature	E. Ratcliffe	Date	10/12/2018

1. INTRODUCTION

1.1 Background & aim of project	The project aim is to study the biocompatibility of 3D-printed scaffolds comprised of polycaprolactone (PCL), poly(glycerol sebacate) (PGS) and Bioactive glass (BG) for cardiac patch applications. Viability of C2C12 murine skeletal myoblasts will be investigated after seeding onto PCL, PCL-PGS and PCL-PGS-BG scaffolds.
1.2 Description of experimental procedures	C2C12 adherent cells will be cultured under standard conditions in culture flasks. Cells will be split at approximately 60% confluency and seeded at a density of 1-2,000 cells/cm2. Once a sufficient number of cells have been cultured, C2C12s will be seeded onto 1cm x 1cm 3D-printed patches of PCL, PCL-PGS or PCL-PGS-BG. These scaffolds will be fabricated in the materials department and sterilised using 70% ethanol prior to use. The cell-seeded scaffolds will be cultured in a 24-well plate for the duration of the experiment. A PrestoBlue cell viability assay will be carried out at predetermined time points and absorbance values measured to determine cell viability.
1.3 Where will this work be carried out?	Rooms/areas: H25
	Building(s): CBE

☑ **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)
C2C12 Cells	Muscle	Murine	Sigma, UK

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

2.4 Has any material listed in 2.2 been genetically modified in any way? If Yes, add a reference number and complete the GMO Risk Assessment Form.	○ Yes ◉ No
☐ 2.5 Has any of the material listed in section 2.2 been identified in the list of cross-contaminated / misidentified cell lines?	○ Yes ◉ No
2.6. Describe what infectious/communicable disease agents or diseases this material(s) has been screened for, eg HIV, HBV, HCV, TSEs, HTLV etc. *If Yes, provide details*	◉ Yes ○ No
☐ 2.7. Will any clinical history or veterinary screening be provided?	
2.8 What is the likelihood of infection of any of this material? *Consider the worst case if multiple materials are to be used.*	The risk is: ○ High ◉ Low ○ Medium ○ None
2.9 Name and classify the biological agents this material could be infected with	Material Type: N/A
	Agent: N/A
	ACDP / Defra Classification.: N/A
2.10 Describe the type and severity of the disease that can be caused to humans or animals by each of the agents that could be present	N/A

☐ **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. CLASSIFICATION OF HAZARD GROUP

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
3.2. 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	C2C12 cells will be cultured under standard conditions - 37C, 5% CO2
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	35
	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 will be followed. PPE will be worn at all times including lab coat, gloves, closed shoes, shoe covers, and safety glasses when there is a risk of chemical splash.	SOP037 SOP038
5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☑ Yes ○ No	Flasks and plates containing cultured material will be transported between BSC and incubator, ensuring that lids are properly closed to prevent spills. Hands will be wiped after spraying ChemGene to ensure that they are not slippery when handling material. Lab will be monitored for any potential trip hazards in transportation route.	
5.3. Will this material (including waste) be transported locally between sites on campus but outside the laboratory?	☑ Yes ○ No	Waste that is contaminated with biological material will be autoclaved and then transported outside of the laboratory to the bin storage area. Waste bags will be transported in secondary containers to prevent spillages occurring should the bag become damaged. The cells will not be transported between sites on campus.	SOP003
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		

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.5. Will this material be received from organisations elsewhere in the UK or abroad?	☑ Yes ☐ No	The C2C12 cells will be ordered from a UK supplier who provide a certificate of analysis upon purchase. Upon delivery, the package will be checked for any signs of damage, and if any damage is identified , will not be accepted and instructed to return to sender. All cardboard packaging will be removed before goods are brought into the lab, and once secondary packaging has been removed, items will be checked that they are labelled correctly and in good condition. Cells will then be transferred to a quarantine location in the cryostorage bank and a material receipt checklist completed to submit with relevant documentation for quality manager approval before use.	SOP008
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, including visor and cryogloves. Cells will then be placed/removed quickly to ensure there is no damage to other cells in the rack when removed from the liquid nitrogen.	SOP031 SOP013
5.7. Will infectious material be centrifuged?	☐ Yes ☑ No		
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 checked before adding vessels, and any irregularities will be reported to the lab manager. Flasks and plates will be kept closed to prevent spills. If any spills occur, these will be cleaned immediately with 70% IM. When adding or removing flasks, care will be taken not to upset any other user's cultures.	SOP124
5.9. Are sharps to be used at any stage during this activity?	☑ Yes ☐ No	Pipettes will be used during cell culture, which will be placed in the autoclavable yellow sharps container for safe disposal. Sharps containers will not be filled more than three quarters full and will not be placed in waste bags as they can puncture bags and cause injuries. If a sharps injury occurs, the wound will be washed with soap and water, and the lab manager and safety officer informed. The incident will also be reported to occupational health, and an accident report 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	Liquid nitrogen will be 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 when in the lab	SOP037

Control Measure	Details		Reference to SOPs / other documentation
6.2 What type and where will they be stored?	Nitrile	In Lab and in Changing Area	SOP037
6.3 When will laboratory coats be worn and what type are these?	At all times when in the lab	White Howie	SOP037
6.4 Where will lab coats be stored and what are the arrangements for cleaning or disposal?	Stored in the first change	Lab coats must be cleaned at least once a month, or if cont	SOP037
6.5 Provide details of any other types of PPE to be used?	Shoe covers will be worn at all times in the lab, with enclosed shoes. Safety glasses will be worn when there is a risk of splashing from pouring chemicals.		SOP037
6.6 Describe the lab hygiene facilities available and where they are located	Sinks and eye wash stations	In change rooms and labs	SOP037
6.7 Where are the first aid boxes and emergency spill kits located?	First aid kit - CBE office	Biological spill kits - change rooms and autocla	

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 virkon treated for 24 hours, then discarded to the drains with copious amounts of water.	☑ Yes ○ No	SOP003 SOP025
☑ Solid waste	Solid waste contaminated with biological agents, which does not contain any chemicals can be autoclaved on cycle 4 and disposed of via the orange waste stream. If solid waste contains any disinfectant or chemicals, this is not suitable for autoclave and 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 SOP054
All cycles have been validated for the actual load types used? *(If Yes, documentary evidence of the validation must be available)*		☑ Yes ○ No	SOP054
The successful completion of every load is checked prior to disposal?		☑ Yes ○ No	SOP054

7.3 How will liquid waste be disposed of?

☑ To drain?	Autoclaved or virkon treated liquid waste can be poured d	○ Yes ☑ No	
☐ 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)

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☐ Sharps contaminated with cytotoxic or cytostatic material		
☐ Human body parts, organs, including blood bags and blood preserves and excreta that have been pretreated before leaving the site		Disinfection or sterilisation in the lab site > Yellow/Orange lidded rigid one way sealed tissue bins > clinical waste disposal (incineration) *Human tissue waste must be placed in separate containers from non-human waste and labelled 'HTA waste'
☐ 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 use Monthly checks Serviced after 100-150 hours of use.	Weekly	With each use	SOP047
☑ BSCs	Weekly	Before and after each use	Before each use	SOP004
☐ Fume Hoods				
☑ Autoclaves	Inspected before each use	Weekly	Before each use	SOP025
☑ Incubators	Weekly	Weekly or fortnightly	With each use	SOP124
☑ LN2 Stores	Twice weekly	Twice weekly	Twice weekly	SOP013
☑ Freezers	6 montly	6 monthly	6 monthly	SOP016
☑ Fridges	6 monthly	6 monthly	6 monthly	SOP016
Others				
☐ 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?

Name of researcher	Had Training	Date training completed (or will be completed)	If no, state why	+

Name of researcher	Had Training	Date training completed (or will be completed)	If no, state why	+
Bridie Catchpole	(•) Yes ( ) No	9 Oct 2018		x

☐ 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

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	x
When and whom to report the incident	Contact first aider and report incident to lab manager and DSO. Inform	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 material is secure	(✓) Yes ( ) No	Labs can only be accessed by other authorised users who have undergone safety training.	

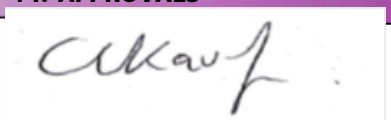
12. OCCUPATIONAL

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		**12/12/18**
Departmental Biological Safety Advisor		