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:	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	Sotiris Korossis	Name	Sotiria Toumpaniari
Position	Professor	Position	Research Associate
Department	Centre of Biological Engineering	Department	Centre of Biological Engineering
School	Wolfson of MEME	School	Wolfson of MEME

The Project Activity			
Title	Use of endothelial cells derived from human induced pluripotent stem cells for tissue engineering applications.		
Reference Number			
Start Date	19 Jul 2021	End Date	31 Aug 2023

Others involved in the work	
Names	Maria Pavlidou
	Sotiris Korossis

Name	Signature	Date
Sotiria Toumpaniari	Sotiria Toumpaniari Digitally signed by Sotiria Toumpaniari Date: 2021.07.09 08:48:09 +01'00'	

1. INTRODUCTION

1.1 Background & aim of project	Endothelial cells (ECs) derived from human induced pluripotent stem cells (hiPSCs)are going to be used to promote endothelialisation of medical devices and will also be used for tissue engineering applications.
1.2 Description of experimental procedures	hiPSC-derived ECs are going to be expanded. Passaging cells -This will involve aspirating media off the cells, washing them gently in PBS and detaching them from the T75 or T125 or T175 flask using Accutase and incubating in a CO2 incubator for 1.5-2 minutes. EGM-2 culture medium with penicillin/ streptomycin will be added immediately following incubation to quench the trypsin reaction and the cells will be transferred to a sterile centrifuge tube. The cell suspension will be centrifuged at 300xg for 5 minutes. The supernatant will be removed to waste and the cell pellet will be re-suspended in fresh supplemented EGM-2 culture medium. The cells will be counted using NucleoCounter. Following calculation of viability, cells will be seeded into new culture flasks that are coated with fibronectin. Cells will be used up to passage number 12. Feeding cells - Medium will be removed from culture flasks and replaced with fresh supplemented EGM-2 culture medium flasks will be return immediately to the 5% CO2 incubator. Freezing cells - A working cell bank will be prepared in accordance to standard procedures as detailed in SOP031 "Cryopreservation and Storage of Mammalian Cell Lines". Freezing medium that is consisted of 10% DMSO with 90% FBS will be prepared and 1 ml cell suspensions will be added to labeled cryovials before placing them at -80 degrees C. Cells will remain at -80 degrees C for 24 and then, they will be transferred to liquid nitrogen. Thawing vials - Vials will be thawed in accordance to standard procedures as detailed in SOP032 "Resuscitation of Cryopreserved Mammalian Cell Lines". Vials will be removed from storage and placed in a 37 degrees C water bath before being transferred to the BSC and added dropwise to 9ml warmed supplemented EGM-2 culture medium. Cell suspension will be centrifuged at 1200 rpm for 5 min before being resuspended in fresh supplemented EGM-2 culture medium and placed in a 5% CO2 incubator. All procedures will be conducted in accordance with lab QMS requirements, good cell culture practice, good aseptic techniques, the local CBE Code of Practice (COP) and the university biological safety policy.
1.3 Where will this work be carried out?	Rooms/areas: H27, H25, H23
	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)	+
hiPSC-derived ECs	Cord blood	Human	Hannover Medical School, Hannover, Germany	x

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

Government Human Tissue Authority - Web Page

☐ **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	HG1	

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	hiPSC-ECs will be cultured on fibronectin coated surfaces under static conditions.
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	10,000,000
	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	Aerosols may be generated when manually pipetting or manipulating solutions. A class 2 BSC will be used for all open manipulations to protect cell line from contamination and to ensure any aerosols generated are contained. BSCs will be operated in accordance to SOP009 "Use and Maintenance of Herasafe KS Class II BSC) or SOP104 "Use and Maintenance of HERASAFE KS Class II re-circulating BSCs" depending on which BSC is being used.	SOP009 "Use and Maintenance of Herasafe KS Class II BSC) or SOP104 "Use and Maintenance of HERASAFE KS Class II re-circulating BSCs"
5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☑ Yes ○ No	Cells will be contained in sealed flasks and sealed secondary containers if being transported within the laboratory. In the event of an accidental breakage, resulting in a biological spill, this will be cleaned up immediately according to SOP038 "Biological Spill Response".	SOP038 "Biological Spill Response"
5.3. Will this material (including waste) be transported locally between sites on campus but outside the laboratory?	☑ Yes ○ No	Transport outside CBE lab unit is highly unlikely, any movement is likely to be constrained within the University campus in sealed flasks and sealed secondary containers with outer packaging and using local procedures: SOP038 "Biological Spill Response"	SOP038 "Biological Spill Response"
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	Transportation is unlikely, but if required, cells will be packaged in sealed containers within secondary containment vessels with the appropriate hazard labels used. Approved couriers will be used when required.	SOP005 - Storage & Transport of Biological Agents v2
WHO guidance for transport of infectious substances website			
5.5. Will this material be received from organisations elsewhere in the UK or abroad?	☑ Yes ○ No	Cells will be packaged in sealed containers containing dry ice within secondary containment vessels with the appropriate hazard labels used. Approved couriers will be used when required.	SOP005 - Storage & Transport of Biological Agents v2

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.6. Will this material be stored?	☑ Yes ○ No	Any vial will be removed from the N2 stores by an authorised user according to SOP013 "Use and Maintenance of Liquid Nitrogen Stores" Any further cell stocks will be stored within -80˚C freezer, in sealed vials and secondary containment, located in the store room (H18) within CBE lab unit.	SOP013 "Use and Maintenance of Liquid Nitrogen Stores"
5.7. Will infectious material be centrifuged?	☑ Yes ○ No	Sealed rotors and buckets will always be used. Sealed Buckets will be opened upon bench top, unless a spillage within a bucket is suspected, in which case the buckets will be transferred to a BSC and opened within a controlled environment. The following SOPs will be strictly adhered to: SOP088- "Use and Maintenance of Sigma 1-14 Microcentrifuge" SOP308- "Biological Spill Response" Biological spill kits are readily available in each laboratory change room or directly inside laboratories that do not have change rooms.	SOP111 "Use and Maintenance of Sigma 1-14 Microcentrifuge", SOP308- "Biological Spill Response"
5.8. Are biological samples to be cultured in an incubator?	☑ Yes ○ No	Static 5% CO2 37˚C Incubator Leaks and/or spillages will be dealt with according to approved CBE SOPs which specifically detail methods to prevent, contain and respond to leakages and spillages in an incubator	SOP053- "Use and Maintenance of the Sanyo MCO-18AIC Incubator" SOP038- "Biological Spill Response" SOP114- "Use and Maintenance of the Heracell CO2 Incubators"
5.9. Are sharps to be used at any stage during this activity?	☑ Yes ○ No	Glass slides will occasionally be used. Occasionally needles will need to be used to remove reagents (such as DMSO) from sealed bottles. In these cases users will never re-sheath needles. Sharps will be disposed of in either cytotoxic sharps bins (when using chemicals) or autoclavable bins for biological materials only.	CBE code of practice, SOP088, 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	Oxygen sensors that activate alarm when oxygen levels are low/	SOP013 "Use and
	☐ 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 laboratory. Glove will be changed at all appropriate times at regular intervals	CBE code of practice, SOP037, SOP038

Control Measure	Details		Reference to SOPs / other documentation
6.2 What type and where will they be stored?	Nitrile	In Changing Area	CBE code of practice, SOP037
6.3 When will laboratory coats be worn and what type are these?	At all times in the laboratory	White Howie	CBE code of practice
6.4 Where will lab coats be stored and what are the arrangements for cleaning or disposal?	In changing area	Monthly clean by lab manager	SOP037
6.5 Provide details of any other types of PPE to be used?	Laboratory safety glasses will be worn as directed by relevant SOPs when working within the CBE. Face shield (primarily for handling liquid nitrogen) will be worn when retrieving cell vial from storage in the CBE as directed by SOP013 "Use and Maintenance of Liquid Nitrogen Stores" Full length aprons will be worn when retrieving cell vial from liquid nitrogen stores in the CBE facility.		SOP013 "Use and Maintenance of Liquid Nitrogen Stores" and when operating the autoclave as directed by SOP025 "Use and Maintenance of Systec VX-95 Autoclave CBE045
6.6 Describe the lab hygiene facilities available and where they are located	Designated eye wash stations and hand washing facilities are available in the change room of each laboratory; other hand basins are situated directly inside the analytical laboratory and in the main change area as entering and exiting the facility.	Changing room outside lab.	SOP038 - Biological spill response
6.7 Where are the first aid boxes and emergency spill kits located?	Chemical spill kits are located	All biological spill kits are in the changes rooms	

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	Virkon Decontamination according to SOP003 "Disposal of Biological Waste"	☑ Yes ○ No	SOP003 "Disposal of Biological Waste"
☑ Solid waste	Samples with seeded cells will be treated in 1% Virkon solution and after 24 h the Virkon and samples will be disposed according to SOP003.	☑ Yes ○ No	SOP003 "Disposal of Biological Waste"
☐ Other (Specify)			
7.2 Is any waste being autoclaved?		☑ Yes ○ No	SOP003 "Disposal of Biological Waste", SOP025 "Use and Maintenance of the Systec VX-95 Autoclaves"
All cycles have been validated for the actual load types used? *(If Yes, documentary evidence of the validation must be available)*		☑ Yes ○ No	SOP025 "Use and Maintenance of the Systec VX-95 Autoclaves"
The successful completion of every load is checked prior to disposal?		☑ Yes ○ No	SOP025 "Use and Maintenance of the Systec VX-95 Autoclaves"

7.3 How will liquid waste be disposed of?

7. WASTE

☑ To drain?	After treatment with virkon	◉ Yes ○ No	After 1% Virkon decontamination for 24 hours, waste is poured down the drain followed by copious amounts of water. Refer to SOP003 "Disposal of Biological Waste" In the occurrence of a contamination, flask will be treated with 3% Virkon overnight before being disposed of, refer to SOP003 "Disposal of Biological Waste"
☐ 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	**Purple**	Yellow/Purple lidded Sharps bin >clinical waste disposal (incineration @ 1000C)
☐ 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	**Purple**	Yellow/Purple clinical waste bags > clinical waste disposal (incineration)
☑ 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	Weekly inspections carried out during lab clean. Serviced every 2 years	Performed according to relevant SOP		SOP004 – General laboratory housekeeping SOP088- "Use and Maintenance of Sigma 1-14 Microcentrifuge
☑ BSCs	Weekly inspections carried out during lab clean. Serviced every 12 months.	Before and after every use the BSC is wiped down with 1:50 chemegene, which is left to dry then followed by 70% IMS. There is a thorough weekly clean with 1:20 Chemgene which is left to dry then followed by 70% IMS.	Alarms are present on the BSCs to inform if the sash is not correctly positioned. The display in the BSC also detailed the level of air flow which is monitored and recorded on every use.	SOP009- Use and Maintenance of Herasafe KS Class II BSC SOP104- Use and Maintenance of HERASAFE KS Class II re-circulating BSCs SOP004 – General laboratory housekeeping

8. MAINTENANCE

☐ Fume Hoods				
☑ Autoclaves	6 months	Autoclaves have weekly and monthly cleaning as detailed in SOP. The usage is recorded each time it is used and whether issues occurred.	The autoclave alarms when a cycle fails	SOP025 "Use and Maintenance of Systec VX-95 Autoclave CBE045" SOP024 "Use and Maintenance of Systec VX-95 Autoclave CBE044
☑ Incubators	Inspection during weekly lab duties. Annual servicing.	Decontamination is accordance with SOP.	Alarms triggered for incorrect temperature and CO2 concentration	SOP053 "Use and Maintenance of the Sanyo MCO-18AIC CO2 Incubator"
☑ Liquid N_2 Stores	LN2 stores are checked and topped up twice weekly		O2 alarms are in place any time that LN2 stores are being refilled. LN2 stores are connected to temperature probes to monitor storage temperatures.	SOP013 – Use and maintenance of liquid nitrogen stores
Failure contingency plan				
☑ Freezers	-Inspected / defrosted and cleaned every 6 – 12 months -Monthly temperature checks with a calibrated thermometer along with other inspections and manual challenge of alarms	2% Neutracon/ 1% Virkon followed by 70% IMS	On board alarms and thermocouples linked to monitoring system.	SOP016 "Use and maintenance of Fridges and Freezers"
Failure contingency plan				
☑ Fridges	Inspected / defrosted and cleaned every 6 – 12 months	2% Neutracon/ 1% Virkon followed by 70% IMS	On board alarms and thermocouples linked to monitoring system.	SOP016 "Use and maintenance of Fridges and Freezers"
Failure contingency plan				
☑ Others	Nucleocounter NC-3000			SOP121 "Use and maintenance of Chemometec NC3000 Nucleocounter"

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	+
Sotiria Toumpaniari	◉ Yes ○ No	16 Jan 2019		x
Maria Pavlidou	◉ Yes ○ No	15 Oct 2019		x
Sotiris Korossis	◉ Yes ○ No	15 Mar 2019		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

10. EMERGENCY PROCEDURES

☑ Within the BSC	SOP006- Selection and Use of Virkon, SOP009- Use and Maintenance of Heras
☑ Within the centrifuge	SOP088- "Use and Maintenance of Sigma 1-14 Microcentrifuge" SOP308- "Bio
☑ Within the laboratory, but outside any primary control measures (e.g. BSC)	1 - SOP006- Selection and use of Virkon Disinfectant 2- SOP038- Bioloigcal Sp
☑ Outside the laboratory	SOP038 "Biological Spill Response". Spill responses are detailed in SOP005 - S

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- flood area with running water plus soap and water. Face- flush with eye wash for 15 minutes, flush eyeball for 15 mins with cold water, hold eye open. For breakages to skin- encourage bleeding, do not suck. Ingestion- contact first aider. In the event of a serious injury requiring medical attention, individuals should attend the Accident and Emergency Department/Minor Injuries Unit of the local hospital.	Ref to SOP's	CBE SOP038 "Biological Spill Response"	x
When and whom to report the incident	Immediately to laboratory management and first aiders. University on	Ref to SOPs	CBE SOP038 "Biological Spill Response"	

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	Access to CBE laboratories is restricted to authorised users. In order to obtain authorised user status, operators must satisfy minimum training requirements set by CBE management and Health and Safety Committee. Basic training modules include a detailed review of the current Code of Practice (CoP), this document details specific aspects of class 2 working in relation to handling biological agents, waste management, training requirements of lab equipment and emergency procedures including spill responses. All training is documented in a personal training file, which is held in the CBE office at all times. Prior to being granted access to CBE labs, each training file must be reviewed and signed off by both lab management and the departmental safety officer (DSO). Once authorised access has been granted, it is the responsibility of the operator to identify specific training needs prior to the start of new projects. SOPs and risk assessments relevant to project equipment and/or procedures can be used as training aids. Training files are live documents and	CBE code of practice, SOP004

11. ACCESS

		must be continually updated to record all training acquired. Restricted access to laboratory. Swipe card access and key rights are given only to authorised personnel that have undergone training and have filled appropriate risk assessments. Unauthorized personnel has no access.	
11.3. Describe the measures in place to ensure that hazardous biological agents or **HTA relevant** material is secure	☑ Yes ○ No	Biological material will be decontaminated after experiment by immersing it in 1% Virkon for 24h. If storage is required material will be stored in PBS with 1% P/S at 4°C. Restricted access to laboratory. Swipe card access and key rights are given only to authorised personnel that have undergone training and have filled appropriate risk assessments. Unauthorized personnel has no access.	SOP005, SOP003

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	Professor Sotiris Korossis Digitally signed by Professor Sotiris Korossis Date: 2021.07.12 11:59:17 +01'00'
Departmental Biological Safety Advisor	Kulvindar Sikand Digitally signed by Kulvindar Sikand Date: 2021.08.06 14:59:06 +01'00'