Example BUA Using Cell Lines

|  |
| --- |
| **Project Description** |
| New application or renewal of BUA # | New application |
| **Project Title** |
| Engineering materials for 3D cell culture |
| **Project Objectives** Provide a brief summary of the goals of the proposed research. |
| Our lab seeks to develop novel 3D cell culture platforms to mimic specific features of the native extracellular matrix. We are interested in how cells interact with and influence their microenvironment broadly, and particularly how mechanical cues of the extracellular matrix alter cell phenotype. To validate these artificial matrices and explore new molecular mechanism, we will culture cells, including those of human origin inside these hydrogels.  |
| **Materials and Methods**Characterize the biological materials and summarize the experimental procedures used in the project. A detailed step-by-step protocol is not necessary, but provide sufficient information so that the Biosafety Committee may assess the risks and identify the steps that involve the greatest likelihood of researcher or environmental exposure. Include culture volumes and whether any steps are conducted in a biosafety cabinet.  |
| 1. Routine culture of human mesenchymal stem cells, or cancer cell lines of human origin. Includes passaging, cell counting, freezing, cry storage, and thawing. Cell counting will be performed on a dedicated Beckman Coulter Multisizer adjacent to the tissue culture room. Cells will be frozen in a shared -80 deg C freezer and transferred to a shared liquid nitrogen dewar in room 2208. All other steps will be conducted in a biosafety cabinet.
2. Encapsulation of these cells in 3D hydrogel matrices. These gels are made by mixing cells with the uncross linked polymer solution, and then mixing the solutions with a cross linker in a tube, well plate, or luer lock-coupled syringes (without needles) and depositing into a culture dish. Occasionally, biopsy punches will be used to cut several small gels from a larger one. These will be deposited into a biohazard medical waste sharps container.
3. Cells will be cultured in these gels and then assessed based on specific projects. Common assays include fluorescence microscopy, including on live cells (using cytocompatible fluorescent dyes, not virally modified), western blots, RNA extraction and PCR, and library preparation for next generation sequencing.
4. Hydrogel materials are sterilized by filtration and then brought into the biosafety cabinet for encapsulation. Once cells are encapsulated, all work is performed in the BSC or the cells are stored in an incubator until fixation (except live-cell imaging, see below).
5. Cells will be imaged on a confocal microscope with a commercial live-cell incubation chamber that will be setup in Bio Engineering Room 2216. Cells will be transported in secondary containers that can be disinfected.
 |
| **Risk Assessment – Biological Materials and Infectious Agents** Specify the known and suspected biohazards of the biological materials or infectious agents, including hazards to health adults, pregnant women, immunocompromised individuals, or other species. * If applicable, specify the symptoms post-exposure or infection in people.
* Include a one- or two-sentence review of lab acquired infections involving the agents you plan to use.

If you are using an exotic arthropod vector system or any plant pathogen, discuss the possible consequences of a release into local agricultural areas or natural ecosystems. |
| All cells are purchased commercially (ATCC) and have tested negatively for HIV, HBV, HCV, HPV, EBV, and CMV. We will purchase (MCF10A (CRL-10317), MCF7 (HTB-22), MDA-MB-231 (HTB-26), mouse D1 ORL UVA (CRL-12424)). We will also purchase from Lonza (human mesenchymal stem cells (PT-2501)), and these cells are screened for HIV-1, hepatitis B, and hepatitis C. Thus, these cells lines will be handled with BSL2 practices, but will not be considered medical waste.No symptoms from exposure to these cell lines are anticipated.  |