SOP TITLE:	VERSION:	
PM-PD-SOP2.3 Extracting cells from PeptiMatrix hydrogels	1	
AUTHOR & RESEARCH TEAM:	STATUS:	
Johnathan Curd (JWC) - Product Development	Shared	

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1. BACKGROUND

PeptiMatrix™ hydrogels are formed from short peptide sequences of between 8-20 amino acids. At the correct concentration, pH, and temperature, they self-assemble to form a stable entangled network of nanofibers. This process can also be reversed so that the gels undergo a gel-liquid transition, to extract cells for re-seeding or downstream processing.

2. RISK ASSESSMENT

Please refer to PM-RA0 and PM-RA2 for a full risk assessment of the following protocol. Ensure that you fully understand the potential risks and have received the appropriate training before starting any work.

3. MATERIALS

- Class II Microbiology Safety Cabinet (or appropriate class for your cells/materials)
- Centrifuge
- Centrifuge tubes (e.g., 15 mL falcon tube)
- P1000, P200, P20, P10 filter tips
- P1000, P200, P20, P10 pipette
- Cells encapsulated or seeded on PeptiMatrix hydrogels
- Cell culture media appropriate for your cells

4. METHODS

Cell extraction from a 96-well plate

1. Using a P200 pipette, aspirate 200 µL of cell culture media and insert the end of the pipette tip into the gel, until it is touching the bottom of the well plate.
2. Pipette up and down several times to thoroughly mix the cell culture media with the gel. This will shear the gel and

dilute it until it transitions to liquid phase.
    1. **NOTE:** For high stiffness hydrogels, the gel might not completely transition back to liquid phase, but this can be addressed in the following step.
3. Transfer the total volume of the well to a 15 mL centrifuge tube. Repeat for as many other wells as required.
    1. **NOTE**: For high stiffness hydrogels, the collected volume can now be further diluted with cell culture media to ensure complete transition to liquid phase. Be sure to thoroughly mix any cell culture media added to the collected volume in the same way as in the previous step.
4. The resulting solution can now be treated in the same way as a cell suspension of the cell types(s) in use. For example:
    1. Cells can be pelleted and re-suspended in fresh media for re-seeding (following separate SOP),
    2. Pelleted cells can be combined with various commercially available lysis buffers to extract cell contents for downstream processing (e.g., extraction of DNA for PCR analysis).

Cell extraction from other well plates or inserts

The same basic principal can be applied to gels plated into other sizes of well plates or within inserts.

Notes on hydrogel concentration

PeptiMatrix™ hydrogels are self-supporting only above the critical gelation concentration (CGC) for the peptide(s) used in their manufacture.

Depending on the concentration used during manufacture, a dilution factor of between 1/2 to 1/5 is typically sufficient for complete transition to liquid phase.

5. DISPOSAL

Hydrogels seeded with cells and/or matrix components should be treated in the same way as suspensions of the cell type and matrix components in use.

User Initials Legend

JWC Johnathan Curd
MES Mikayla Shelton