Culturing cells on PolyGEM™ in LeviTube™ v1.0

The following protocol describes how to:

1. Prepare a stock solution of PolyGEM
2. Load cells onto the PolyGEM
3. Maintain cells on the PolyGEM
4. Collect and passage cells with the PolyGEM.

Materials:

- PolyGEM with coating of choice
- Cells of choice
- Cell culture media of choice
- LeviTube

Preparing a stock solution of PolyGEM:

1. Typically the stock solution is 25 mg of PolyGEM per mL. However, depending on your goals, a more concentrated or dilute stock might be preferred.
2. As an example we will make a 25mg/mL stock solution and use 5g of PolyGEM in 20 mL of DI water.
3. Autoclave the solution for 15 to 45 minutes at 121°C.
4. The stock solution can be stored and used for several months if handled aseptically.

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Suggested Starting Volumes per LeviTube™:

Vessel	**PolyGEM** (25mg/mL)	Cells	Media
Levitube	2 mL	4 million	8 mL

* *These volumes are recommended based on our past experiments. However, you may find that different volumes will provide better results for your research. Initial volume of cells can range from 250,000 to 6 million cells, depending on the cell type used.*

Loading Cells on the PolyGEM:

1. Wash the PolyGEM once with media.
2. Add the appropriate volume of media to the culture vessel.
3. Add 2 mL of (25mg/mL) PolyGEM solution to the media.
4. Prepare a cell suspension. Accutase can collect cells from plastic as well as from PolyGEM.
5. Add appropriate volume cells to the media/PolyGEM preparations.
6. Shake gently and place in the BioLevitator™
7. Check and monitor for cell adhesion.

* *Cell attachment and spreading can be observed at 20x magnification on an inverted microscope at various time intervals such as 2, 4, 18 or 24 hours and qualitative assessment of the attachment and spreading can be performed. Cells can be visualized at the edges or circumference of the PolyGEM as rounded (initial attachment phase), "gumdrop-shaped" (early spreading) or flattened (completely spread).*

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Maintaining Cells on the PolyGEM:

1. Media exchange is done in the same manner as traditional cell culture using an indicator such as phenol red.
2. When ready to change media, remove LeviTube from BioLevitator and place on shelf inside a traditional cell culture incubator.
3. Allow PolyGEMs to settle to bottom of the LeviTube, this could take up to 15 minutes.
4. Once the PolyGEMs have settled, gently aspirate the used media leaving a quarter to a third of the media. Leaving some used media will avoid shocking the cells.
5. Add the fresh warm media to bring total volume to 40 ml.

Collecting Cells from the PolyGEM:

1. Remove the LeviTube from the BioLevitator and place on shelf inside an incubator.
2. Allow the PolyGEMs to settle to the bottom of the LeviTube, this can take up to 15 minutes. Carefully aspirate the media.
3. Add 5 ml of PBS. Gently mix by rubbing the LeviTube™ back and forth in your palms as if warming your hands.
4. Once again, allow PolyGEMs to settle and carefully aspirate the PBS.
5. Add 10-20 mL of Accutase. Place the LeviTube™ back into the BioLevitator™. Allow this to mix for 15-20 minutes or until cells detach. Accutase will not damage cells like trypsin.
6. Using a cell strainer (i.e. BD Biosciences 70µm Cell Strainer cat. no. 352350) pipette contents from LeviTube™ through the cell strainer into a 50mL conical tube. The cell strainer will separate the PolyGEMs and allow you to collect your cell suspension.

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Additional Tips:

- *Use Accutase to prepare your cell suspension for maximum viability.*
- *Serum concentrations can be reduced in PolyGEM culture because there is no need to adsorb protein to the culture surface. Serum can be removed all together but a proper serum-free media must be used*

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