2-D Cell Growth on HyStem Hydrogels

This protocol describes how to make HyStem®-C hydrogels in a 96-well plate format for cell growth on the surface of the hydrogels. The protocol can easily be adapted for use with 6-, 12-, 24-, and 48-well plates. It can also be adapted for use with HyStem-HP hydrogel kits, merely substitute all instances of Glycosil with Heprasil.

Required Materials:
HyStem-C or HyStem-HP hydrogel kit
One sterile 96-well plate

Procedure:
1. Solubilize HyStem-C Hydrogel Kit components under aseptic conditions as directed by the instructions.

2. Mix equal volumes of Glycosil® and of Gelin-S®, either 1.0 mL each or 5.0 mL each depending on kit size. Pipette up and down thoroughly to mix.

Note: If the HyStem® solutions are not well mixed, then the hydrogel surface may not be uniform. This can cause variation in how the cells attach and grow on the hydrogel.

3. When you are ready to pour the hydrogels, add one part Extralink® to four parts Glycosil + Gelin-S mix (typically 0.5 mL Extralink to 2.0 mL mixture, or 2.5 mL Extralink to 10 mL mixture depending on kit size). Pipette up and down thoroughly to mix.

Note: Once the Extralink is added you have < 20 minutes before the hydrogel forms and it becomes impossible to uniformly pipette the solution.

4. Pipette 100 µL of hydrogel into each well. Place the lid on the 96-well plate.

Note: Leftover solutions can be frozen at -20°C and are viable for ~2 weeks.

5. Allow the hydrogel to gel by placing the plate on a rocker for at least one hour with the lid on.

Note: If the hydrogel is left for an extended period of time, it will dry out and form a film.

6. Prepare cells for use in 2-D cell culture as per standard procedures.
Seeding density varies with cell type, but a typical range is 5,000 to 50,000 cells per well.

7. Once the hydrogels are solid, add 100 µL of cell slurry in media to each well on top of the hydrogel.

8. Place in the 37°C incubator with 5% CO2.

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Changing Media:

1. Carefully aspirate off the media. The hydrogel can easily be removed by the vacuum, so this must be done gently and carefully.

2. Pipette 100 µL media into each well. Try to avoid disrupting the gel.

3. Return the plate to the 37°C incubator with 5% CO2.

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