Co-culture of Spheroids and Organoids

Protocol

The ClinoStar® system is an *in vitro* system to grow primary and immortal cell lines to retain and recover their *in vivo* physiological features. This protocol describes how to maintain co-culture spheroids or organoids created from primary cells or immortal cells lines in the ClinoStar®. There are numerous ways of co-culturing mixtures of cells therefore individual steps should be optimized accordingly. CelVivo recommend conducting a pilot experiment with different cell ratios in the co-culture mix as cells may replicate at different speeds. There are two approaches to co-culturing: premixed cell suspension causing the cells to self-aggregate, or a coating approach where spheroids created from one cell type are subsequently coated/exposed to a single cell suspension of another cell type. For primary cells self-aggregation is an excellent way to create spheroids or organoids. Often primary cells are obtained from the enzymatic digestion of tissue biopsies and as such contain more than one cell type. Self-aggregation is slower and gives cells extra time to organise themselves and find the correct neighbours. Coating with individual cell suspensions can be useful for co-culturing cells with different growth rates or if a specific structure is desired e.g., Cancer cells coated with fibroblast.

Reagents and Materials

- Cells or biopsy
- ClinoStar®
- ClinoReactor®

Additional information

Preparation of the ClinoReactor® is described in **003_Protocol_Preparation_Of_ClinoReactor**.
Preparation of spheroids or organoids from different procedures are described here:

- Self-aggregation: **008_Protocol_Spheroids_from_single_cell_suspension**
- Microplate aggregation: **009_Protocol_low_adherent_microwell_plate_setup**
- Q-gel aggregation: **010_Protocol_Preparation_of_spheroids_from_Q-gel**
- Alginate aggregation: **011_Protocol_Preparation_of_alginate_encapsuled_spheroids**

Protocol

1. Prepare the required number of ClinoReactors®
2. Cultivate your cells or collect you sample.

Co-culturing with premixed cell suspensions for self-organisation

1. Prepare single cell suspensions and evaluate cell count.
2. If the ratio between the cell suspensions is variable conduct a pilot experiment to find the optimal ratio for your experiment.
3. Select the aggregation method: Self– aggregation, low adherent microwell plate aggregation, QGel aggregation or alginate aggregation.
4. Start cultivating.

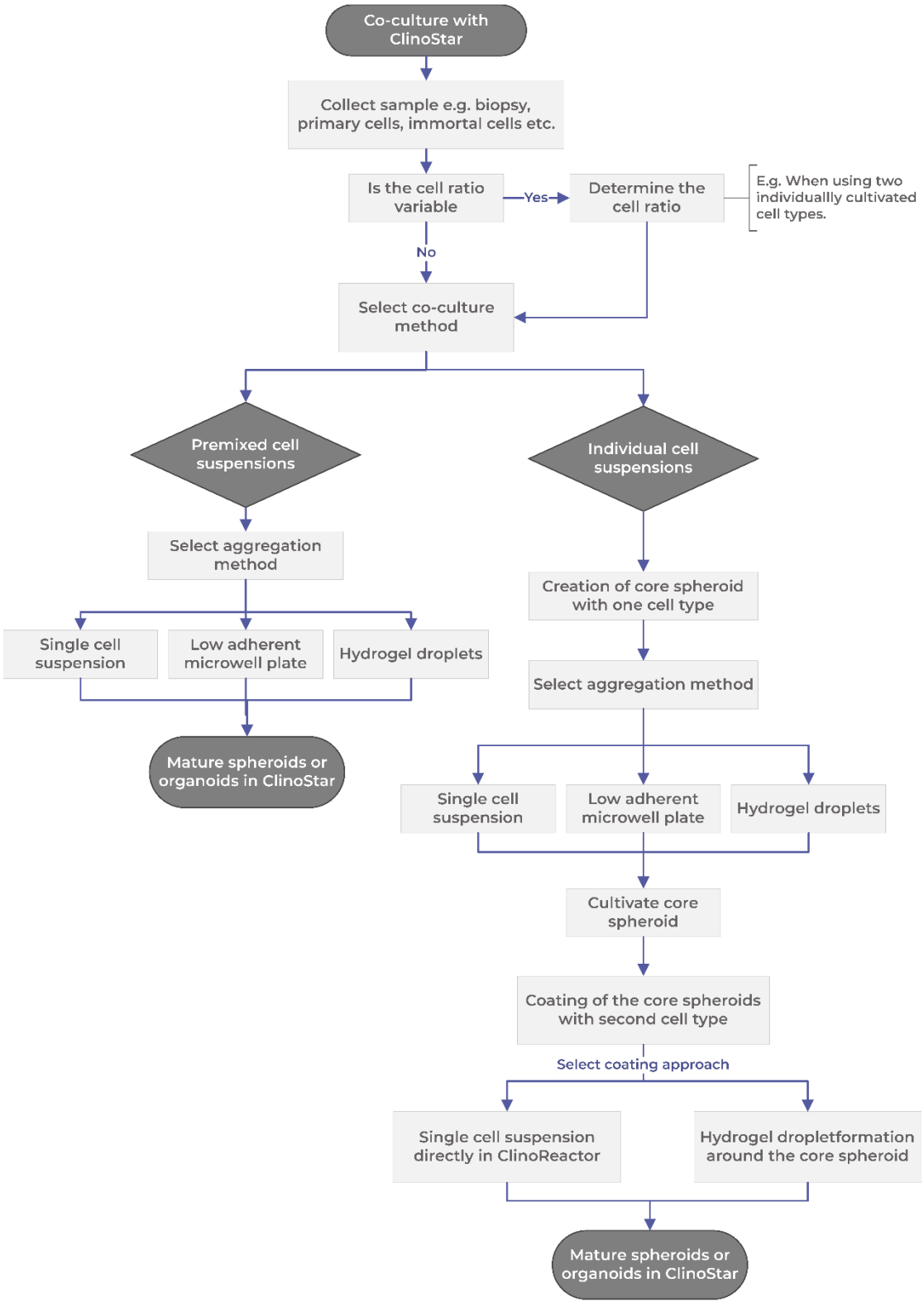
Co-culturing with individual cell suspensions, the coating approach

1. Prepare single cell suspension of the “core” spheroid or organoid.
2. Select the aggregation approach: self– aggregation, low adherent microwell plate aggregation, QGel aggregation or alginate aggregation.
3. Cultivate the core spheroid.

4. Prepare the second cell suspension for coating of the “core” spheroid or organoid.
5. Select coating approach:
    a. Single cell suspension. The second cell suspension is injected directly to the ClinoReactor®
    b. Hydrogel coating. The core spheroids are removed from the ClinoReactor® and coated with a hydrogel suspension containing the second cell type and reintroduced into the ClinoReactor®
6. Start cultivating.

Warranty/disclaimer: This equipment is for research use only. Materials produced by the use of this equipment must not be used for diagnosis or treatment in any type or form.

For additional product or technical information visit www.celvivo.com or consult CelVivo Aps at info@celvivo.com or +45 70 228 228.


Co-culture with ClinoStar
Collect sample e.g. biopsy, primary cells, immortal cells etc.
Is the cell ratio variable
Yes
Determine the cell ratio
E.g. When using two individuallly cultivated cell types.
No
Select co-culture method
Premixed cell suspensions
Individual cell suspensions
Select aggregation method
Single cell suspension
Low adherent microwell plate
Hydrogel droplets
Mature spheroids or organoids in ClinoStar
Creation of core spheroid with one cell type
Select aggregation method
Single cell suspension
Low adherent microwell plate
Hydrogel droplets
Cultivate core spheroid
Coating of the core spheroids with second cell type
Select coating approach
Single cell suspension directly in ClinoReactor
Hydrogel dropletformation around the core spheroid
Mature spheroids or organoids in ClinoStar