BioResearch

Lonza

Determination of the Desired Cell Seeding Density in RAFT™ 3D Cell Cultures

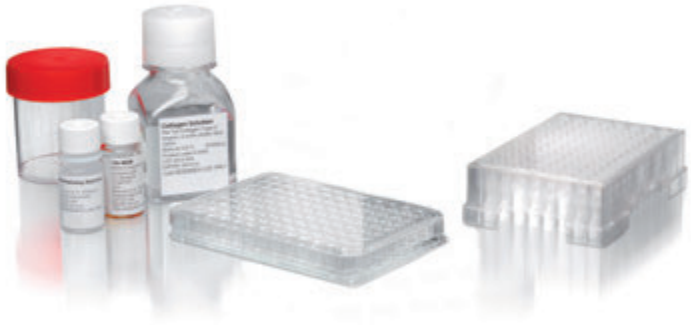
Technical Notes

Introduction

When beginning a new experiment with 3D Cell Culture it can be a vital step to assess the impact that the cell seeding density has on cell behaviour, viability and morphology. It is quite likely that the number of cells per well has to be increased, for instance by a factor of 10 to 50 times the number of cells per well of the same dimension used in a 2D experiment. With the RAFT™ System, this is not a difficult experiment to carry out. In this technical note, we give a few hints and tips to generate a series of RAFT™ 3D Culture densities easily into the same culture plate. In more advanced cases where co-cultures are being investigated more complex mixes may be required, for instance, to study the ratios of cell types and their behaviour. However the principle is the same as outlined below.

Materials

- RAFT™ Absorbers and Reagent Kit
- RAFT™ Instructions downloadable from www.lonza.com/raft under 'product knowledge center'
- For a list of recommended manufacturer cell culture plates (either 96-well black wall or 24-well plate not supplied with the kit), contact Lonza scientific support team
- Cell line(s) or primary cells of interest
- Culture medium for the cells of interest
- Bucket filled with ice
- 15 mL centrifuge tubes or other appropriate sterile containers



Methods

When setting up different cell density cultures, it is easier, more accurate and precise to first make up a collagen master mix (obtained at the end of step 1.6 of the standard protocol).
This collagen master mix can then be divided into different tubes (e.g. 15 mL sterile centrifuge tubes), into each of which the different cell stock solutions can be added.

Example

In this example triplicates of RAFT™ Cultures with 4 different cell densities (e.g. 0, 5,000, 20,000 and 50,000 cells/well) were prepared.
In the table of reagent volumes for different numbers of wells, (see RAFT™ full protocol downloadable from www.lonza.com/raft under the section 'product knowledge center'), it is advised to prepare 1.7 mL of cell seeding collagen mix in order to make 3 RAFT™ Cultures. We therefore wish to create four times that volume for this experiment so make four times this mixture for the master mix, e.g. 6.8 mL.
In the same table, you can see that n=20 makes up 6.8 mL of solution, therefore you can use this to get the correct volumes of each reagent. The reason for this is that the table in the protocol takes account of some wastage of reagents due to their viscous nature; however due to the splitting of the master mix you require additional wastage volume over a single mix.
In a centrifuge tube, make up the master mix by following the standard protocol up to step 1.6. Then, transfer the 1.7 mL for each triplicate into 3 sterile tubes leaving 1.7 mL of master mix in the original RAFT™ Mixing vessel. Keep each tube embedded in ice to prevent premature gelling until you have prepared the different cell stock solutions.
As shown in the table RAFT™ Instructions, you will need 71 µL of cell stock solution at the concentrations indicated in the RAFT instructions sheet. Once the stock solutions with 0, 4.96 x 10^5 cells/mL, 1.98 x 10^6 cells/mL and 4.96 x 10^6 cells/mL are ready, add 71 µL into the different tubes.
Mix the cells in as indicated in the standard protocol. If using a 15 mL centrifuge tube, you can mix the cells by carefully and slowly inverting the tube a number of times to avoid introduction of bubbles into the mix. You can also mix by aspirating and dispensing the mix in the tube prior to transferring the solution into the wells of the culture plate (using the same tip to reduce waste).

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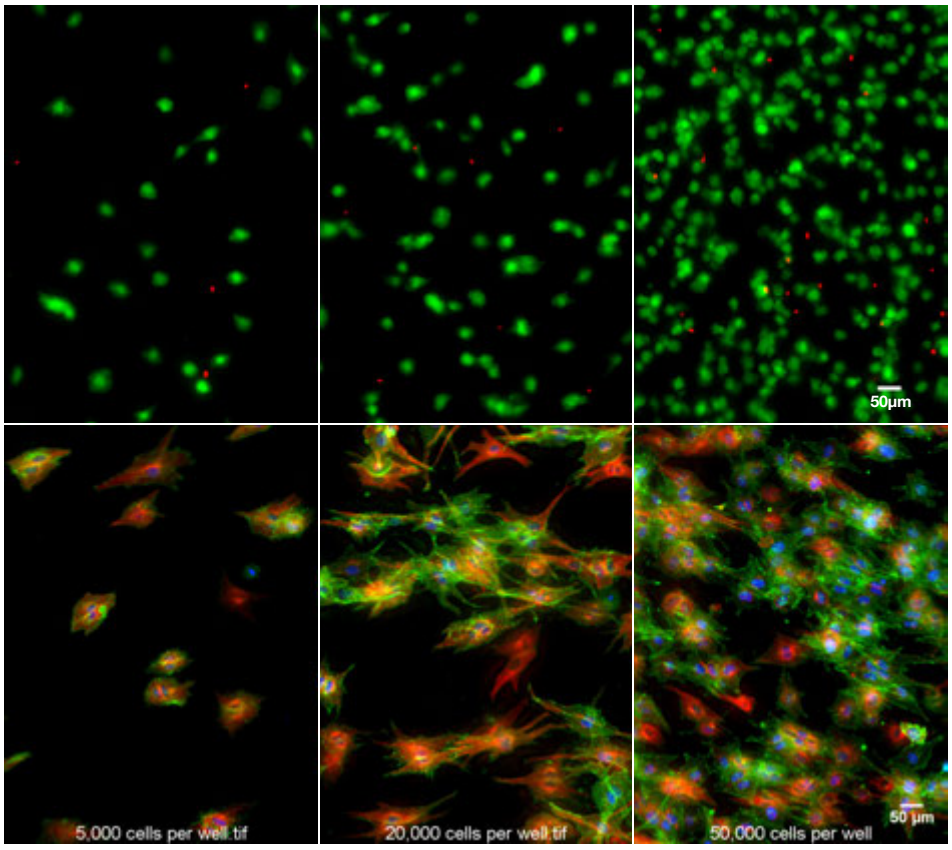

Figure 1: Human dermal fibroblasts, seeded at 5,000, 20,000 and 50,000 cells/well, were cultured in RAFT™ System for 2 or 3 days (top and bottom rows respectively). In the top row, live cells are stained in green using Calcein AM (Life Technologies), while dead cells are stained in red using propidium iodide (Life Technologies). In the bottom row, the cells are stained for tubulin using yol 1/34 antibody (Abcam and secondary antibody from Jackson ImmunoResearch, red), actin using Phalloidin (Life Technologies, green) and nuclei using DAPI (Life Technologies). For each image, a montage with only the focused part of each z-stack slice is shown (each stack was processed using ImageJ software).

Follow the RAFT™ Instructions from step 4 on to finish preparing your RAFT™ Cultures.
Immediately after the making of RAFT™ 3D Cell Cultures and the addition of the appropriate medium it should be possible to see the cells, which are often round at this stage, in the RAFT™ Culture by phase contrast microscopy. However, it should be noted that, due to the z-dimension of the culture, cells are difficult to see and staining of some kind may be required. This is particularly true for cells which elongate.

Conclusions

Cell seeding density in 3D culture can have a profound effect on behaviour of the cells being studied. By following the simple steps in this technical note this determination can be made easily in a single plate.

This Technical Note was generated originally by TAP Biosystems, who developed the RAFT™ Products. Lonza is now the exclusive distributor of RAFT™ 3D Kits.

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