Construction of a 3D hepatocyte model using the RAFT™ 3D Cell Culture System
Therese Willstaedt[1], Lubna Hussain[1], Maureen Bunger[2], Theresa D'Souza[1]
[1]Lonza Walkersville, Inc., Walkersville, MD, USA;
[2]Lonza Research Triangle Park, NC, USA

Lonza
Pharma & Biotech

Construction of a 3D hepatocyte model using the RAFT™ 3D Cell Culture System

The RAFT™ Hepatocyte 3D Model is comprised of primary hepatocytes embedded in compressed type 1 collagen

In this Technical Note, we describe the details of plating primary human hepatocytes in the RAFT™ 3D Culture System in 96-well plates. The RAFT™ Hepatocyte 3D Model is comprised of primary hepatocytes embedded in compressed type 1 collagen. The procedure for optimally producing these 3D models differs from the standard RAFT™ 3D Culture Kit Protocol in that the cell stock solution is four times higher and the volume of collagen hydrogel is four times less. Hepatocyte handling protocol differs from standard methods by maintaining cells in a highly concentrated format following thaw and forgoing the use of a serum containing plating medium. These details are outlined in the protocol below.

Materials

- Cryopreserved Human Primary Hepatocytes (Lonza, Cat. No. HUCPI or HUCPG)
- Human Hepatocyte Thawing Medium (Lonza, Cat. No. MCHT50)
- 96-well tissue culture treated plates (Greiner, Cat. No. 655180)
- RAFT™ Reagent Kit for 3D Culture (Lonza, Cat. No. 016-0R94)
- RAFT™ Absorbers (Lonza, Cat. No. 016-0R92)
- HBM™ Basal Medium (Lonza, Cat. No. CC-3199)
- HCM™ SingleQuots™ Kit (Lonza, Cat. No. CC-4182)
- Trypan Blue (Lonza, Cat. No. 17-942E)
- Hemocytometer
- 50 mL Sterile Reagent Reservoirs (Corning, Cat. No. 4870)

Methods

Note: The following is an abbreviated protocol containing key changes to standard protocols. For additional detailed technical information and instructions for thawing cryopreserved hepatocytes and the use of the RAFT™ 3D Culture Kit, please refer to Lonza Hepatocytes Protocol. and Lonza RAFT™ Protocol respectively.

Preparation of Hepatocyte Culture Medium (HCM)

Thaw HCM™ SingleQuots™ Medium (CC-4182) overnight at 4°C. In a Biosafety Cabinet (BSC), add thawed SingleQuots™ Kit, one at a time using a separate pipet for each growth factor, to a 500 mL bottle of HBM (CC-3199). Swirl to mix thoroughly.

Thawing primary human hepatocytes

Thaw hepatocytes in a 37°C water bath until only a sliver of ice remains. In the Biosafety Cabinet, pour contents into a 50 mL conical tube containing 45 mL warm Hepatocyte Thawing Medium. Collect cells remaining in the vial by pipetting back 1 mL of Thawing Medium and combining with the previous. Centrifuge the cells at 100 x g for 8 minutes at room temperature.

Carefully aspirate most of the supernatant, leaving approximately 100 µL of Hepatocyte Thawing Medium over the pellet to maintain a highly concentrated cell suspension. Gently tap the centrifuge tube against the palm of your hand to resuspend the cell pellet in the remaining Hepatocyte Thawing Medium.

Note: This step creates a highly concentrated hepatocyte cell suspension to enable the formation of the collagen compressed disc.

Counting primary hepatocytes

For counting, remove 4 µL from cell suspension and dilute 1:100 with 396 µL using HCM. Count diluted hepatocytes using Trypan Blue exclusion methods detailed in the Lonza Hepatocytes Protocol. A cell seeding density of 45,000 – 65,000 cells each well per 96-well plate is optimal.

After counting, dilute cells using HCM to the desired cell concentration according to Table 1.

Number of cells per well	Cells/mL
45,000	1.75×10^7
55,000	2.18×10^7
65,000	2.60×10^7

Table 1
Concentration of cells required to achieve various numbers of cells per 96-well.

Preparing the RAFT™-Hepatocyte Hydrogel

The RAFT™ 3D Cell Culture Kit Protocol provides guidance for the construction of RAFT™-Hepatocytes 3D Cultures (Figure 1). The exception to the guidance is that one quarter of the recommended volume per well is used (60 µL of cell seeded collagen hydrogel is used, rather than the standard 240 µL). Use recommended volumes from Table 2 to form the collagen solution using contents of the RAFT™ 3D Cell Culture Kit and the hepatocyte cell suspension based on the desired number of experimental wells in a 96-well plate.

Note: Reducing the volume per well and thickness of the final 3D construct is necessary for maintaining good hepatocyte viability during the culture period.

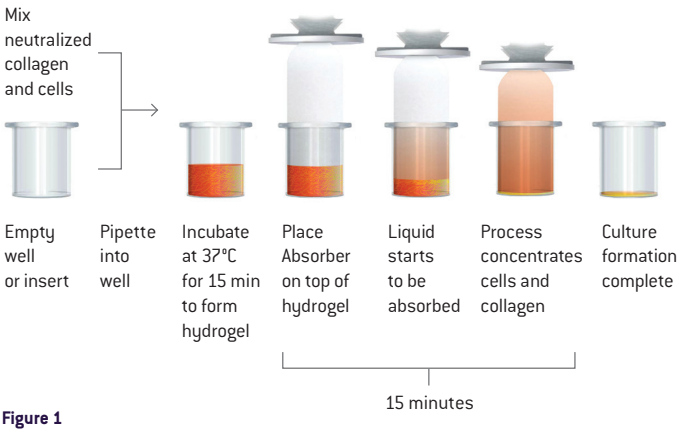


Figure 1

The RAFT™ 3D System Process. Cell containing RAFT™ Cultures are formed within less than one hour.

96-well plate (No. of wells desired)	10X MEM (mL)	Callogen (mL)	Neutralizing solution (mL)	Hepatocyte suspension (mL)	Total (mL)
10	0.08	0.71	0.049	0.036	0.88
12	0.1	0.85	0.059	0.043	1.05
48	0.40	3.4	0.236	0.170	4.21
96	0.80	6.8	0.472	0.340	8.41

Table 2

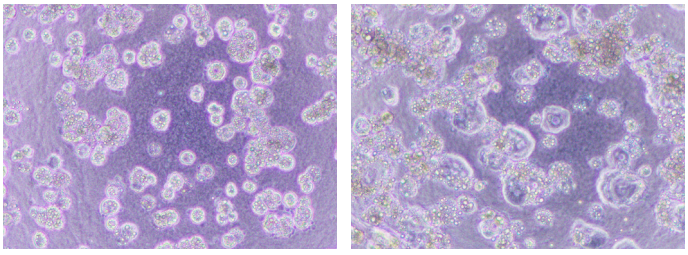
Concentration of cells required to achieve various numbers of cells per 96-well.

Mix the chilled collagen solution containing hepatocytes gently by swirling, avoiding introduction of bubbles, and pipet 60 µL into the center of each well and replace plate lid after seeding. If dispensing using a multichannel pipet, pour the cell-seeded collagen solution into a sterile reagent reservoir. Mix the suspension by gently rocking the reservoir prior to each aspiration of cells.

Allow the hydrogel to form by incubating the plate at 37°C for 15 minutes. Following the incubation, remove the plate lid in the BSC and place the RAFT™ Absorbers on top of the hydrogel by following the guidance provided in the RAFT™ 3D Cell Culture Kit Protocol. Leave for 15 minutes at room temperature in the BSC. Remove the RAFT™ Absorbers after the incubation period and add 100 µL of complete HCM™ to each well.

Note: An incubation step in a serum containing Hepatocyte Plating Medium is unnecessary and does not improve viability or formation of cell-cell contacts. Return the plate to the incubator (37°C, 5% CO_2). Replace the medium daily with warm HCM.

Note: The 96-Absorber plate can be stored aseptically and unused absorbers may be used in future experiments.



Day 0 Day 5

Figure 2

Expected morphology of hepatocytes in RAFT™ Cell Culture.

Contact information

North America

Customer Service: +1 800 638 8174 (toll free)
Fax: +1 301 845 8338
order.us@lonza.com
Scientific Support: +1 800 521 0390 (toll free)
scientific.support@lonza.com

Europe

Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com

International

Contact your local Lonza distributor
Customer Service: +1 301 898 7025
scientific.support@lonza.com

Lonza Walkersville, Inc. – Walkersville, MD 21793

For research use only. Not for use in diagnostic procedures.
All trademarks belong to Lonza or its affiliates or to their respective third party owners. The information contained herein is believed to be correct and corresponds to the latest state of scientific and technical knowledge. However, no warranty is made, either expressed or implied, regarding its accuracy or the results to be obtained from the use of such information and no warranty is expressed or implied concerning the use of these products. The buyer assumes all risks of use and/or handling. Any user must make his own determination and satisfy himself that the products supplied by Lonza Group Ltd or its affiliates and the information and recommendations given by Lonza Group Ltd or its affiliates are (i) suitable for intended process or purpose, (ii) in compliance with environmental, health and safety regulations, and (iii) will not infringe any third party's intellectual property rights.

©2018 Lonza. All rights reserved.
CD-DS033 12/18

www.lonza.com/raft