Application Note

INTEGRA

Cell seeding on BIOMIMESYS®*Hepatocyte* hydrogels with the VIAFLO 96/384 channel handheld electronic pipette

Introduction

Creating three-dimensional (3D) spheroids suitable for high content screening (HCS) may be difficult and is often an expensive process. Here, Celenys – now part of HCS Pharma – and INTEGRA describe how a 96 channel pipette can help seeding and maintenance of a 3D cell culture with BIOMIMESYS® *Hepatocyte* hydrogels in microplates. The method achieves physiologically relevant cellular models in a high throughput screening (HTS) format.

Key benefits:

- The accurate z-height and pipetting speed set-up of the VIAFLO 96/384 handheld electronic pipette enables worry-free cell seeding, removal of culture media and drug compound testing.
- The 96 channel head processes all the microplate wells at once, saving a lot of time. Fewer steps are required compared to a multichannel pipette, which minimizes the risk of errors and improves the reproducibility of cell cultures.
- The small footprint VIAFLO 96/384 fits easily into a laminar flow cabinet, making the device ideal for all kinds of cell culture applications.

BIOMIMESYS hydrogels for 3D culture

BIOMIMESYS is a range of new patented hyaluronic acid scaffolds for 3D cell culture. Celenys scaffold is made of RGDS and galactosamine-grafted hyaluronic acid, adipic acid dihydrazide crosslinker, and extracellular matrix (ECM) proteins (collagen type I and IV). BIOMIMESYS is suitable for robotic tests due to the thickness of the scaffold – around 600 µm – with an average porosity ranging from 100 to 200 µm (see **Figure 1**).

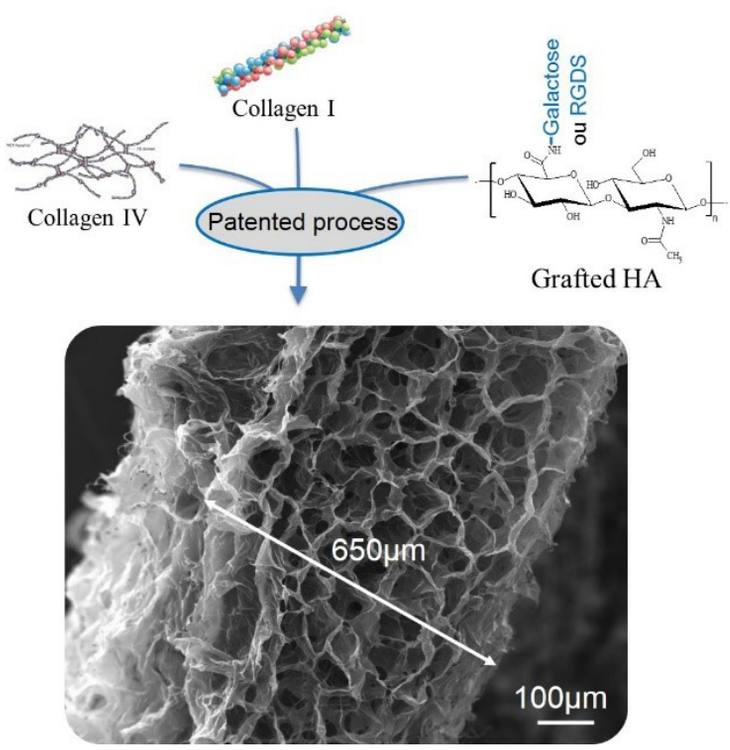

Figure 1: BIOMIMESYS synthesis and SEM observation of a section of the final hydrogel product

INTEGRA

Step-by-step procedure:

Experimental set-up

The VIAFLO 96/384 is used for seeding and renewing cell medium. The device is equipped with a 96 channel 300 µl pipetting head and 300 µl Sterile, Filter GripTips are used. The entire application is performed in a laminar flow cabinet.

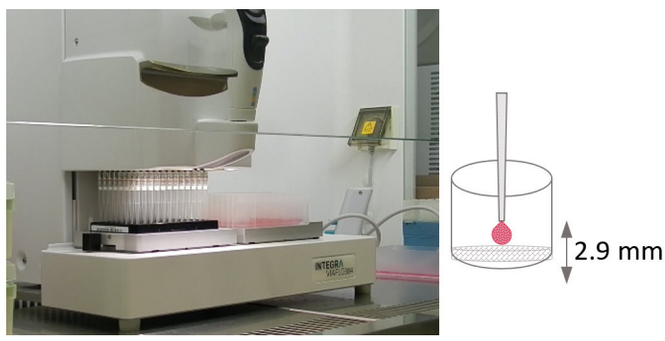

Figure 2: HepG2 seeding on a 96 well plate containing BIOMIMESYS *Hepatocyte* hydrogels using a 96 channel head on the VIAFLO 96/384

1. Cell seeding on BIOMIMESYS hydrogel with the VIAFLO 96/384	**STEP:** Homogenize HepG2 cells and seed on the ready-to-use BIOMIMESYS *Hepatocyte* hydrogel. This step takes less than two minutes.	**HOW TO:** HepG2 cells are homogenized in an automation friendly reagent reservoir (150 ml or 300 ml) by aspiration and dispensing. This is achieved using the Pipet/mix mode of the VIAFLO 96/384. Then, cells are transferred into a 96 well plate containing the BIOMIMESYS *Hepatocyte* hydrogel. Selecting the right height (z-height limit: 2.9 mm) and pipetting speed (set on 2) settings on the VIAFLO 96/384 is essential to guarantee homogenous seeding of the cells on the hydrogel (see **Figure 2**). Cell medium (150 µl) is added by setting the z-height limit to 3.2 mm and the pipetting speed to 2.
2. Chlorpromazine treatment of HepG2 cell line	**STEP:** Remove culture medium and add the hepatotoxicity-inducing drug chlorpromazine.	**HOW TO:** Carefully remove 100 µl of medium and add 100 µl of the drug, using a pipetting speed of 1 to prevent harming the cells. A z-height limit of 3 mm is set to ensure spheroids are not accidently aspirated or destroyed by the pipette tip.
3. HepG2 viability assay	**STEP:** Determine the metabolic activity of HepG2 cells using a WST-1 assay.	**HOW TO:** Remove 100 µl of culture medium using the normal Pipet mode at speed 2 and a z-height limit of 3 mm. Add 10 µl of the WST-1 reagent using the same pipette settings described in step 2.
4. Biliary canaliculi activity of HepG2	**STEP:** Biliary canaliculi activity of HepG2 cells treated with chlorpromazine was examined using 5(6)-carboxy-2′,7′-dichlorofluorescein diacetate (CDFDA) (21884, Sigma).	**HOW TO:** Remove 100 µl of culture medium and add 200 µl of PBS to the HepG2 cells, using the normal Pipet mode with the same z-height and speed described in step 2. Wash the HepG2 cells by adding and removing 200 µl of PBS (repeat this step five times). The convenient three position stage of the VIAFLO 96/384 accommodates the waste and reagent reservoirs (**positions A** and **AB**, respectively) needed for the washing step, and a target sample plate (**position B**).

Conclusion

- Easy liquid handling, time savings and high accuracy in generating identical data sets.
- Very low variability in cellular metabolic activity between wells.
- Treatment, seeding and viability assay are all performed with the VIAFLO 96/384.
- Easy observation using a microscope.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6317	150 ml, 30 Reservoirs, Individually Sealed, Sterile, Polystyrene	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	6001/6031	VIAFLO 96 or 384 handheld electronic pipette (base unit)	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6103	96 channel pipetting head 300 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6215/6220	Spring loaded plate holders	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6435	300 µl Sterile, Filter, GripTips	https://www.integra-biosciences.com/global/en/griptip-selector-guide
HCS Pharma	N/A	Hepatocyte plate	https://biomimesys.com/products/biomimesys-liver/

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