INCREASED CULTURE TIME

INTRODUCTION

BIOMIMESYS®*Liver* recreates the complex cellular microenvironment of the liver to extend longevity and functionality of hepatocytes in culture:

- The life of human primary hepatocytes grown in a 2D sandwich is low, 3-10 days (1) and is accompanied by dedifferentiation, leading to the loss of function.
- In 2D culture, HepG2 due their ability to proliferate quickly, reach confluence and have low hepatocyte functions limiting their use for metabolic studies or drug induced liver injury, DILI (2)

Therefore, a culture in BIOMIMESYS® *Liver*:

- Doubles the human primary hepatocytes viability compared to a 2D culture.
- Allows HepG2 to grow without impairing the viability and morphology for at least 4 weeks.

(1) 3D cultivation techniques for primary human hepatocytes, Bachmann A. et al. Microarrays. 4:64-83, 2015
(2) Comparison of primary human hepatocytes and hepatoma cell line Hepg2 with regard to their biotransformation properties. Wilkening S et al. Drug Metab Dispos. 31:1035-1042, 2003.

Materials required

- BIOMIMESYS®*Liver*
- HepG2 from ATCC & cryopreserved primary human hepatocytes
- Live/Dead®kit (Life Technologies)
- HCM BulletKit (Lonza)
- Brigthfield Microscope
- Epifluorescence microscope

Hydroscaffold properties

Translucent and porous

Method

- Perform Live/Dead® test with the kit, following the manufacturer's instructions

RESULTS

❖ HepG2 grown in BIOMIMESYS®*Liver:*

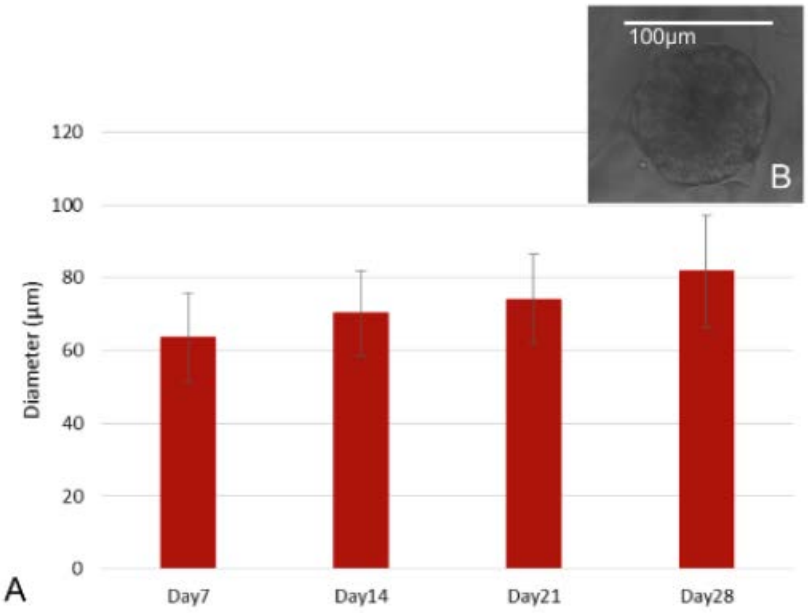

Figure 1: Average size (A) for 28 days (3 independent experiments, n = 70 spheroids measured per experiment) and observation by brightfield microscopy(B) of HepG2 grown in BIOMIMESYS®Liver

HepG2 form spheroids with an average diameter of 80-100 µm after one month of cultivation.

❖ Cryopreserved human hepatocytes grown within the specific matrix

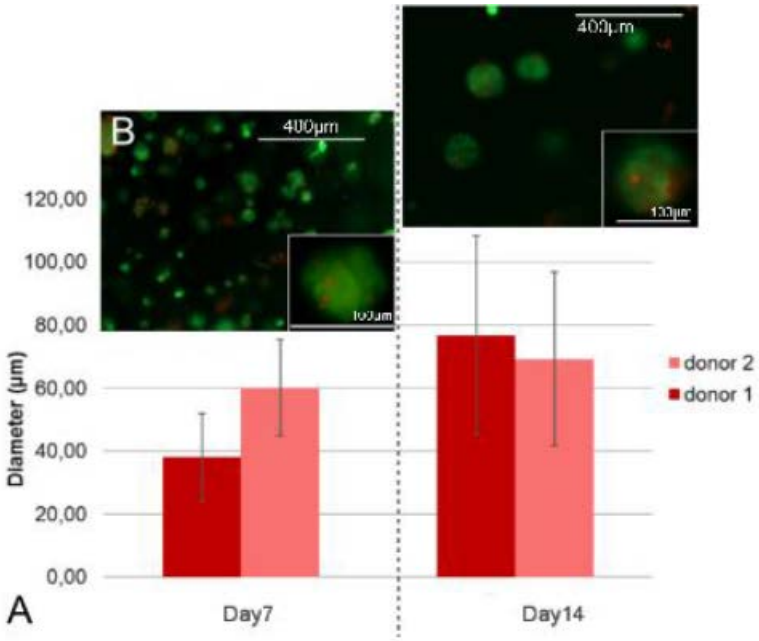

Figure 2: (A) Average diameter of cryopreserved human hepatocytes cultured in BIOMIMESYS®Liver (n ≥25) and (B) observation by fluorescence microscopy highlighting the structure aggregates, at day7 and 14.

Cryopreserved primary human hepatocytes form small aggregates, 40-80 µm, which have an excellent viability until day14.

CONCLUSION

The cells exhibit excellent viability during culture time, greater than those achieved in 2D:

- Two weeks for cryopreserved human hepatocytes, hence up to twice more than in 2D culture with the sandwich technique (1).
- Four weeks for HepG2 cultures.

Furthermore, there is no confluence or cellular degeneration problem with the HepG2 cell line in BIOMIMESYS®*Liver* cultures.

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