Development of a Culture Process to Grow a Full Liver Tissue Substitute

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Introduction

- The liver is an essential multi-task organ and medicine cannot support patients when functional liver volume drops below 20-30% of the total one. This fact has motivated the scientists to find alternative treatments instead of transplantation.
- Cell therapy is one of the ongoing and promising approaches [1].
- Aims: To develop a culture process to grow a full-liver tissue substitute using engineering approaches.
- In this project, isolation and culture of full-liver biopsies have been performed either from mice or, when available, from patients undergoing liver resection (*Biobanque des maladies digestives du CRCHUS following strict ethical guidelines*), rinsed with saline solution and further used to establish in vitro cultures.
- Static cell isolation: After mechanical dissection and incubation under agitation in a proprietary enzymatic formulation following several steps, the resulting tissues are cultured in a growth medium.
- Dynamic cell isolation: The Extra Cellular Matrix (ECM) is digested by perfusion of the digestive medium through the vasculature. The remaining tissue contains a lot of cells.

Results

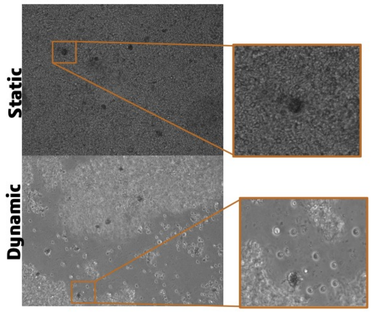
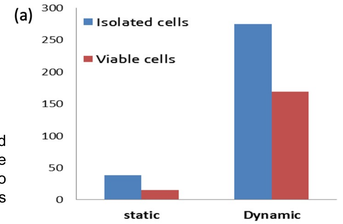
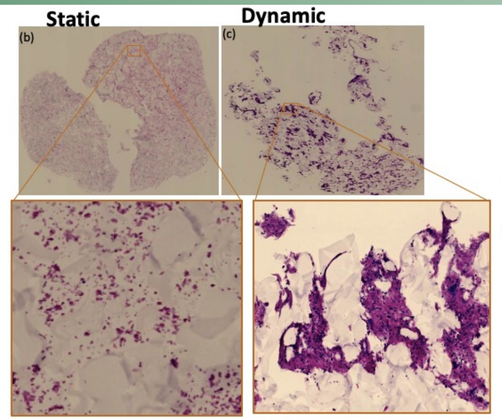


Figure 2: The anti-coagulation effect of EDTA on Red Blood Cells (RBCs) [2]. The residual RBCs are more present in static processed samples, in comparison to dynamic ones. Perfusion through the vasculature is more efficient in removing RBCs.

Figure 4: A significant difference is observed when comparing the number of isolated cells by static and dynamic methods. Perfusion is more efficient, allowing sufficient collagenase penetration within the liver, which is one of the most vascularized organs **[3]**. Also, because of lower exposure time of digestion in dynamic method, higher cell viability seems better (a). H&E micrographs reveal more nuclei in the dynamic processed samples (b, c).





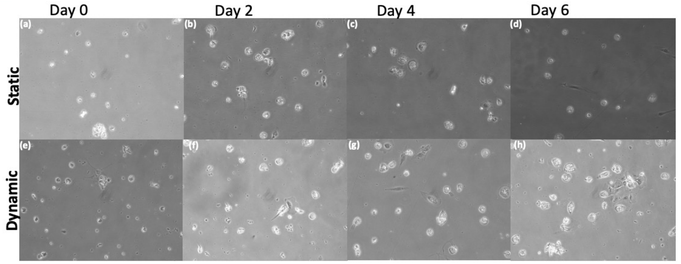


Figure 3: Morphological changes of cells over 6 days are evident for static (a-d) and dynamic (e-h) processed cells.
Although cells were seeded with similar density, a lower cell density is observed in static samples during the culture. Cells are not fully healthy, and some detach during the first PBS rinsing step (a, b).
The attachment of some cells is evident after 2 days, although most cells have remained rounded in morphology **[4]** (b, f).
The cells are not populated over the time indicating this fact that hepatocytes are not capable of being sub-cultured 2D and need a proper 3D microenvironment to proliferate.

Methodology

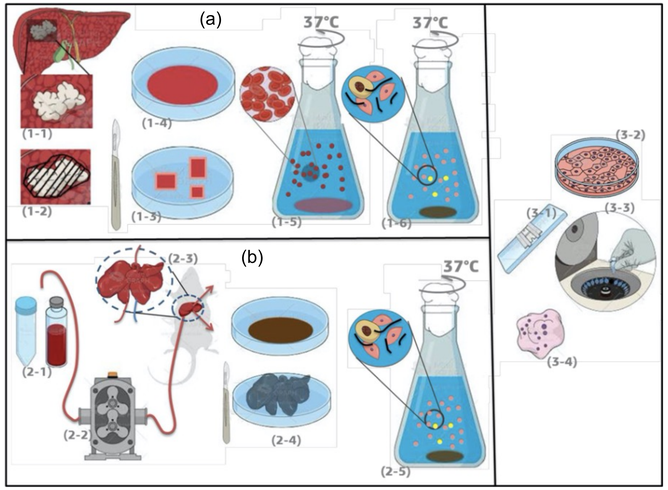


Figure 1:

(a) Static cell isolation technique :

- Human tissues afforded by Biobanque are used.
- (1-1) As the surgeon dissects a tumor, it is accompanied by healthy parts.
- (1-2, 1-3) Those parts are separated, and (1-4) fully minced by scalpel (we have used dissected lobes of rat liver to make a comparison with the next method).
- (1-5) The biopsy is rinsed to wash out the reside of blood cells.
- (1-6) The blood-free biopsy was digested in Dulbecco's Modified Eagle Medium (DMEM) containing 0.08% collagenase II under agitation at 37°C.
- Every 15 min the cell containing supernatant solution was taken stabilized by DMEM+20% FBS growth medium (repeated 3X).

(b) Dynamic cell isolation technique

- Rat tissues are used.
- Because of accessibility to the full vasculature of the liver, a perfusion digestion method was employed.
- (2-1) Similar buffer and digestion solutions were prepared.
- (2-2) A peristaltic pump circulated the solutions through the liver via (2-3) cannulated vena cava.
- (2-4) After 6 min rinsing by buffer and digestion solutions the ECM is fully digested and the organ is easily minced.
- (2-5) The resulting minced tissue was agitated by the growth medium at 37°C (15 min).

After both methodologies (3-1) the cells were counted by a hemocytometer. (3-2) 10,000 cells/well was seeded in a TCPS well plates to evaluate the morphology of cells over the time. The growth medium was changed every two days. (3-3, 3-4) Samples were processed for paraffin-embedding, and sectioned at 6 µm. Then the sections were H&E stained. Sections were imaged using a Hamamatsu NanoZoomer S360 microscope (Japan).

Conclusion

- Primary hepatic cell culture is a useful technique that can be widely utilized in basic liver function research as well as in cell therapies in hepatocellular carcinoma.
- Two methods of cell isolation were developed and compared.
- Preliminary results indicate that the perfusion-based dynamic method is more efficient.

Acknowledgments

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