

Hepatic













Microfluidic

Bioreactor

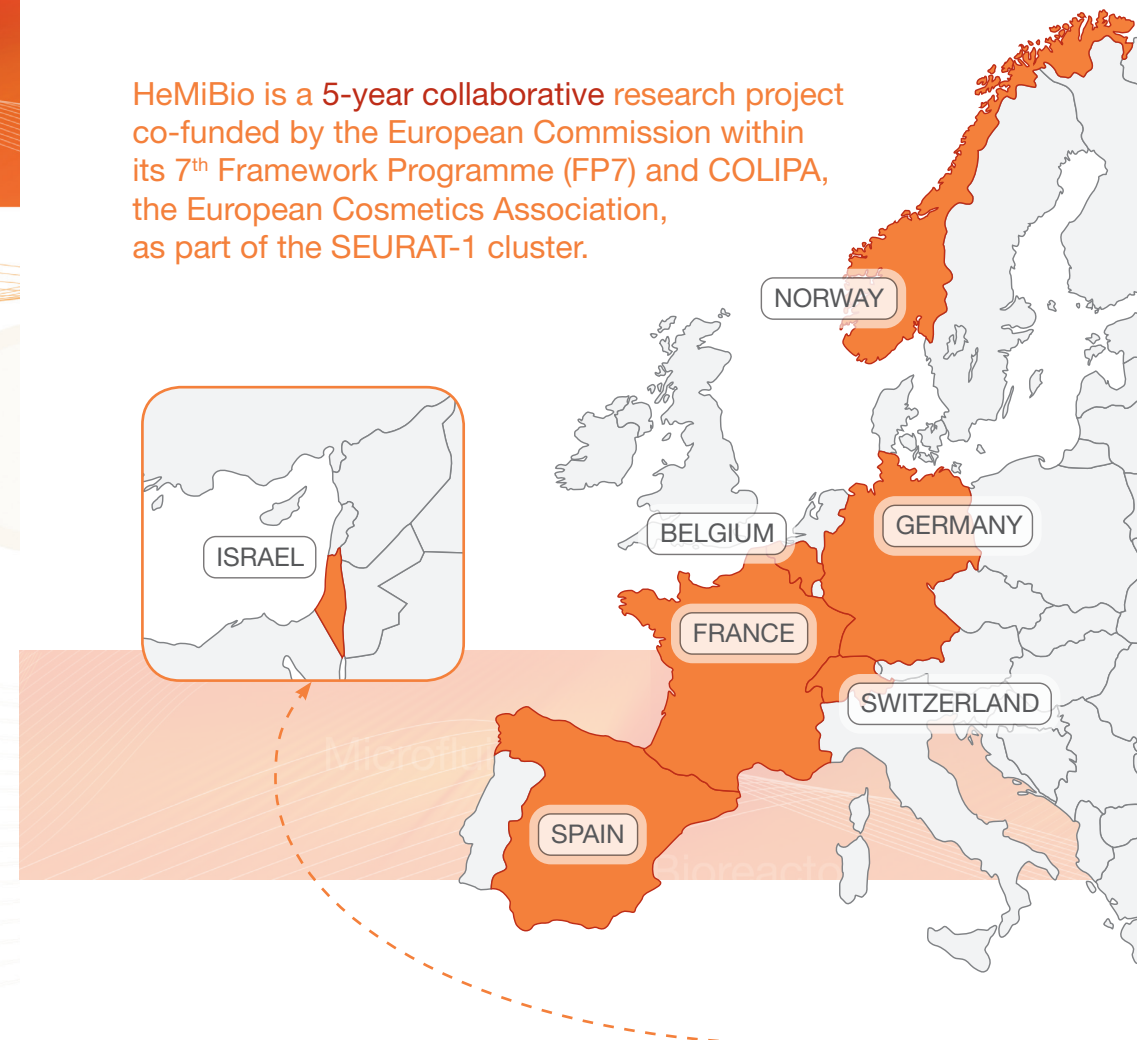
## PARTNERS

The **HeMiBio consortium combines the complementary expertise** of ten internationally renowned academic research laboratories from Belgium, Norway, Spain, Israel, Germany and Switzerland and one small and medium enterprise (Medicyte GmbH).

The consortium recruited world experts with outstanding knowledge of stem cells, cellular and molecular (“-omics”) aspects of hepatocytes and non-parenchymal liver cells, gene editing, liver physiology and toxicity, as well as microfluidics, sensor development and bioreactor assembly.

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HeMiBio is a 5-year collaborative research project co-funded by the European Commission within its 7<sup>th</sup> Framework Programme (FP7) and COLIPA, the European Cosmetics Association, as part of the SEURAT-1 cluster.



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# HEPATIC MICROFLUIDIC BIOREACTOR

“Generate a liver-simulating device mimicking the complex structure and function of the human liver to substantially reduce the use of animals for toxicity testing”



HeMiBio is funded by the European Commission within its FP7 Programme and the European Cosmetics Association (COLIPA), as part of the SEURAT-1 cluster. Grant Agreement number HEALTH-F5-2010-266777.



## CONTEXT

**Refinement, Reduction and Replacement of the use of animals in toxicity tests** is of particular importance for the biotechnology, pharmaceutical and cosmetics industry in Europe. Although important efforts have been made to decrease the need for animals in toxicity testing, the assessment of toxic effects of chronic exposure still requires the use of a relatively high number of animals. Moreover, aside from these ethical considerations, there is also a great need for suitable human cells to be used in toxicity testing, due to the often poor concordance seen between animal models and toxic effects in humans.

**The European Commission and European Cosmetics Association (CO-LIPA)** have thus jointly launched in January 2011 a European research initiative called SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing) that includes six European collaborative research projects aiming at a common strategy "towards the replacement of current repeated dose systemic toxicity testing in human safety assessment".

**HeMiBio is one of the projects funded under the SEURAT-1 cluster umbrella**, with the specific aim of developing a device that simulates the complex structure of the human liver, thus providing the pharmaceutical and cosmetic industry with a standardised tool for preclinical toxicity testing. The HeMiBio consortium should accomplish this challenging objective thanks to the collaboration of outstanding inter- and supra-disciplinary research teams that will work closely together during 5 years.

## Photographs legends

- A - Undifferentiated induced pluripotent stem (iPS) cells from skin fibroblasts.
- B - ESC-derived hepatocytes (Albumin (green) and Cyp3a4 (red)).
- C - Endothelial cells (VE-cadherin in red) and hepatocytes (HNF4alpha in green) from pluripotent stem cells induced for hepatic differentiation.
- D - Activated hepatic stellate cell (Myosin IIA (green), Beta-actin (red), Nucleus (blue)).

## OBJECTIVES

**The aim of vHeMiBio is to generate a liver-simulating device (Hepatic Microfluidic Bioreactor)** mimicking the structure and function of the human liver. The device should reproduce the heterotypic interactions between the parenchymal (hepatocytes) and non-parenchymal (hepatic stellate cells and hepatic sinusoidal endothelial cells) of the liver for over one month *in vitro*, with *in vivo*-like metabolic and transport function. The Hepatic Microfluidic Bioreactor could then serve to test the effects of chronic exposure to chemicals, including cosmetic ingredients, thus limiting the need for animal models.

**To mimic the liver function**, many increasingly more complex and clinically relevant approaches are currently used. However, these approaches are not satisfactory due to the shortage of human livers, as well as the fact that primary hepatocytes rapidly de-differentiate under standard conditions. Hence, what is needed for the cosmetics and pharmaceutical industry are innovative culture systems that incorporate hepatocytes as well as non-parenchymal liver cells, derived from expandable /renewable cell sources. HeMiBio seeks to address this unmet need as the cocultures generated by the consortium will allow induction and maintenance of mature hepatocyte, hepatic stellate cell and hepatic sinusoidal endothelial cell function, while creating a bioreactor that can provide clinically relevant information on drug and chemical clearance and toxicity. This will allow testing of repeated dose toxicity for several weeks to ultimately months.

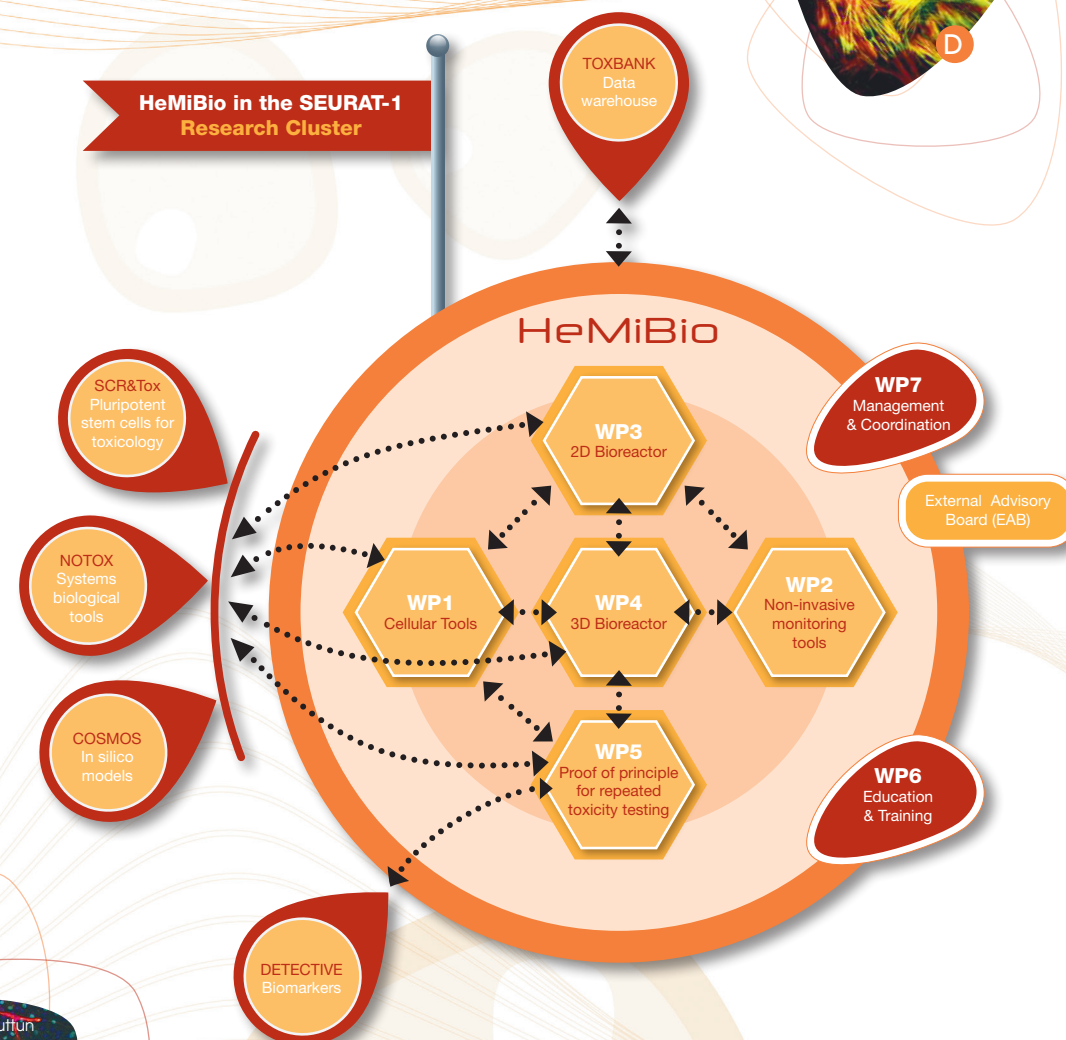
**HeMiBio will be tightly associated to the other consortia of the SEURAT -1 research cluster**, sharing biological, technological and methodological resources, with the common objective of generating important new knowledge that can be genuinely exploited by the biotechnology, pharmaceutical and cosmetic industry in their aim to reduce the use of animals for toxicity testing.

## STRATEGY

**To achieve the creation of a liver-bioreactor, HeMiBio partners will have to go through different steps:**

- Develop tools to engineer three different liver cells** (hepatocytes, hepatic stellate cells and hepatic sinusoidal endothelial cells) generated from induced pluripotent stem cells (iPSC) (or expanded using the UpCyte® technology) to be used in the hepatic bioreactor
- Incorporate molecular sensors** to dynamically measure cell function and toxicity in a high-throughput format
- Develop a 2D-bioreactor** for the efficient isolation of differentiated iPSC mixtures by trapping different cell types on micropatterned surfaces
- Generate a 3D liver-simulating device** mimicking the human liver, which reproduces the function of the hepatocyte and non-parenchymal liver cells over one month in culture. This will be accomplished by combining the above-mentioned engineered cells and sensors under conditions characterised in the previous step
- Provide proof-of-principle** that a liver-simulating device can recreate the toxicity profile *in vitro* of toxins with a known *in vivo* toxicity profile over a minimum of one month, including the barrier function of the liver and the effect of inflammatory (and immune) cells in this process
- Assess the molecular, functional and metabolic phenotype** of the hepatocellular, hepatic stellate and hepatic sinusoidal endothelial cell components at all stages of bioreactor development and compare this with that of cells isolated fresh from human livers

### HeMiBio in the SEURAT-1 Research Cluster



## EXPECTED OUTCOMES

- HeMiBio will significantly decrease the need for the pharmaceutical and cosmetics industry to use primary animal derived cells** for a number of aspects of ADME/Tox studies, and hence REDUCE significantly the need for animals in experimentation as it will allow REPLACEMENT of animal derived liver cells by *in vitro* generated liver cells
- Hepatocytes being the predominant target of HBV and HCV infections** (hepatitis B & C viruses), HeMiBio may alleviate the problem of paucity of human hepatocytes to use *in vitro* to evaluate the infection process, the influence of hepatitis viruses on cell function, and identify additional targets for drug development
- HeMiBio liver-simulating device will allow the evaluation of antifibrotic drugs**: the generation of a renewable source of hepatic stellate cells from hiPSC should make the investigation of possible novel antifibrotic agents more feasible.
- The hepatocytes generated within HeMiBio could be used in Bio-Artificial Liver (BAL) systems**. A BAL device is an artificial extracorporeal supportive device for individuals suffering from acute liver failure that replaces all failing liver functions of the diseased liver, including removal of toxins, thus yielding improved survival. The current systems under development use cell lines that do not show all required liver cell functions. The availability of non-transformed human hepatocytes, whether derived from liver tissue or stem cells would alleviate such problems.
- Translation of HeMiBio results to other organ-simulating devices**. The technology developed in HeMiBio, i.e. cells that are manipulated as such that their differentiation state, functionality and viability can be monitored and the inclusion of sensors that can monitor the environment of the cells, can be translated to other organ systems for high-throughput screening for the effect of drug candidates without the need of animals.