thus time and costs associated with animal handling. Data also
ation. Moreover, undifferentiated rLECs can be easily cryopre
interindividual differences are a significant source of data vari
ibility is a feature that is crucial for industrial application, since
IVTD’s sequential differentiation methodology. The reproduc
of functional hepatocyte-like cells when cultured according to
cannalicular origin) proved a reliable and reproducible source
rat liver
human- and animal-derived stem cells. Specifically,
technique has been tested using diverse sources of postnatal
 growth factors.

genitors by sequential exposure of the cells to hepatogenic
in vitro
primary hepatocytes.

differentiated hepatic cell line by the use of an innovative
developing a fully
-lik phenotype of primary hepatocyte cultures is now
in vivo
options for the treatment of acute and chronic liver
diseases.

drug development process. Additionally, the IVTD group
including CYP1A1 and CYP2B1/2-dependent activity, similar to
indicate that the rLECs-derived hepatocytes acquire a normal
inherent plasticity and differentiation potential towards a specific tissue can be estimated, and a
 adverse effects consti
more than 20 years of experi
alternatives that may be explored (fig. 2). The IVTD team is also
characterization and differentiation of stem cells from
including bone marrow, skin, adipose
tissue modeling
• alternative 3R methods*
• hepatotoxicity
Major research interests of the IVTD group include:
ence in the field of liver-based in vitro modeling.
The IVTD group represents
of drug development costs.
increasing assay speed and throughput, would allow reduction
projecting new drugs which could better reflect human toxico-
going causes. Moreover, animal tests are costly, remain a sub
 toxic potential of new drug candidates is one of the underly-
Therefore, reducing the use of animal-derived hepatocytes
is a major bottleneck in the drug development pipeline. The
Fig. 4: Different tissue sources of adult stem cells (IVTD groups expertise
in vitro models to enable more accurate prediction of
 develop functional liver-based
Fig. 1: Immunofluorescence analysis of Oatp4 DAPI expression in rLECs-derived hepatocyte-like cells after 21 days of hepatogenic differentiation (left) and rLECs-derived hepatocyte-like cells after 21 days of hepatogenic differentiation (right).
Fig. 3: Summary of classical and novel approaches used to maintain primary hepatocytes in culture
Expertise & Techniques

The In Vitro Toxicology and Dermato-cosmetology (IVTD) research group, headed by Prof. Vera Rogiers, is specialized in the field of in vitro experimental toxicology. In essence, the IVTD team strives to develop functional liver-based in vitro models to enable more accurate prediction of the hepatotoxic potential of new drug candidates and alike. IVTD constitutes one of the core groups of the Centre for Pharmaceutical Research (CePhaR) of the VUB, which aims at tackling key bottlenecks in the current drug development process. Additionally, the IVTD group endeavors to find new drug targets and cell therapy options for the treatment of acute and chronic liver diseases.

Located at the medical campus of the VUB in Brussels-Jette, the IVTD group has fully equipped cell culture facilities, a biosafety level 2 laboratory and an organ perfusion unit at its disposal. Moreover, an array of molecular biology techniques has been mastered to evaluate (stem) cell plasticity, functionality, proliferation potential, cell death and adverse effects of drugs. These techniques include gene-specific and genome-wide gene expression profiling, including toxicogenomics, diverse immunoassays, as well as spectrophotometry, fluorometry and radioactivity measurements.

Major research interests of the IVTD group include:
- in vitro toxicology
- hepatotoxicity
- liver disease
- alternative 3R methods*
- in vitro tissue modeling
- cell therapy and epigenetics

* Replacement, Refinement & Reduction - in vitro alternatives

Liver in vitro models: why, what and how?

Retrospective analyses show that adverse effects constitute major stumbling blocks during drug development. Specifically, drug-induced liver injury currently is the main safety-related reason of post-marketing drug withdrawal. The inability of animal-based safety studies to predict the hepatotoxic potential of new drug candidates is one of the underlying causes. Moreover, animal tests are costly, remain a subject of ethical controversy and become gradually prohibited. Therefore, human in vitro cell systems represent a highly desired alternative, which could better reflect human toxicological response, would be ethically more acceptable, and, by increasing assay speed and throughput, would allow reduction of drug development costs.

The IVDT group represents more than 20 years of experience in the field of liver-based in vitro modeling. Initially, the efforts of the team focused primarily on the optimization of cultures of primary hepatocytes, the most prominent liver cell population and the most widely used liver model in pharmaceutical research. Nevertheless, a well-known drawback of primary hepatocytes is their progressive dedifferentiation (i.e. loss of liver-specific functionality) in in vitro setting. To hamper the disadvantageous gene expression changes occurring during hepatocyte dedifferentiation, a novel idea was introduced in 1999, namely the interference with the epigenetic mechanisms of gene regulation i.e. histone deacetylation. In 2002, a parallel strategy was introduced, namely the use of stem cell technology to produce human hepatocytes (fig.3). The obtained hepatocyte-like cells have potential for pharma-toxicological testing, but also have great value for clinical purposes (i.e. cell transplantation).

In Vitro Toxicology and Dermato-cosmetology

Fig. 1: Expression of Organic Anion Transporting Polypeptide 4 (Oatp4), a drug transporter, in undifferentiated rLECs (left) and rLECs-derived hepatocyte-like cells after 21 days of hepatogenic differentiation (right).

Fig. 2: Summary of classical and novel approaches used to maintain primary hepatocytes in culture.
Today, the IVTD research group builds further on its fruitful past. Specifically, the role of other epigenetic mechanisms, such as DNA methylation and microRNAs, in the maintenance of in vivo-like phenotype of primary hepatocyte cultures is now being explored (fig. 2). The IVTD team is also developing a fully differentiated hepatic cell line by the use of an innovative dual genetic–epigenetic immortalization strategy of human primary hepatocytes.

Stem cells-derived models and expertise
With respect to stem cells-based hepatic in vitro models, the IVTD group has developed an innovative protocol to produce hepatocyte-like cells out of various sources of adult progenitors by sequential exposure of the cells to hepatogenic growth factors. In addition, cells are further maturated by the exposure to epigenetic modifiers. The robustness of the latter technique has been tested using diverse sources of postnatal human- and animal-derived stem cells. Specifically, rat liver epithelial cells (rLECs; postnatal stem cells of primitive bile cannalicular origin) proved a reliable and reproducible source of functional hepatocyte-like cells when cultured according to IVTD’s sequential differentiation methodology. The reproducibility is a feature that is crucial for industrial application, since interindividual differences are a significant source of data variation. Moreover, undifferentiated rLECs can be easily cryopreserved and readily expanded according to the needs, reducing thus time and costs associated with animal handling. Data also indicate that the rLECs-derived hepatocytes acquire a normal hepatic morphology and demonstrate hepatic functionality, including CYP1A1 and CYP2B1/2-dependent activity, similar to cultured primary hepatocytes (fig. 1). Both sequential differentiation and rLEC hepaticogenic differentiation methodologies (EP1824965 (B1) and EP2041272 (B1), respectively), as well as rLEC cell bank, are available for out licensing.

Moreover, the IVTD team is highly skilled in the isolation, characterization and differentiation of stem cells from various tissue sources, including bone marrow, skin, adipose and umbilical cord tissues (fig. 4).

In Vitro Toxicology and Dermato-cosmetology
Laarbeeklaan 103 I building G I 2nd floor
1090 Jette (Brussels) I Belgium
Director: Professor Vera Rogiers
[T] +32 (0)2 477 45 16
[E] Vera.Rogiers@vub.ac.be
[W] www.ivtd-fafy.be

Technology Transfer Interface
R&D Department
Vrije Universiteit Brussel
Pleinlaan 2 | B-1050 Brussels | Belgium
[E] rd.interface@vub.ac.be
[W] www.vubtechtransfer.be
[T] +32 (0)2 629 22 07