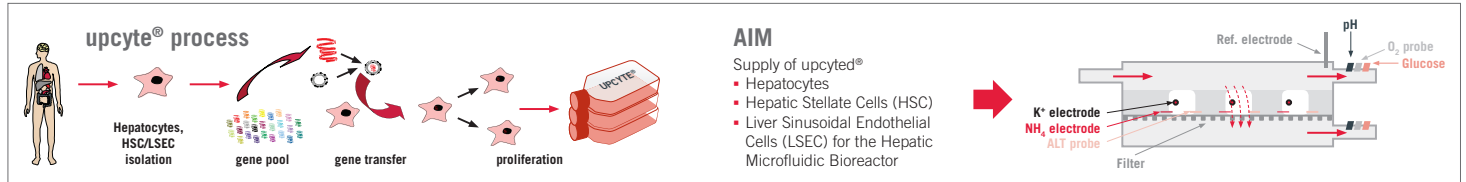


APPLICATION OF UPCYTE® TECHNOLOGY TO LIVER DERIVED CELLS

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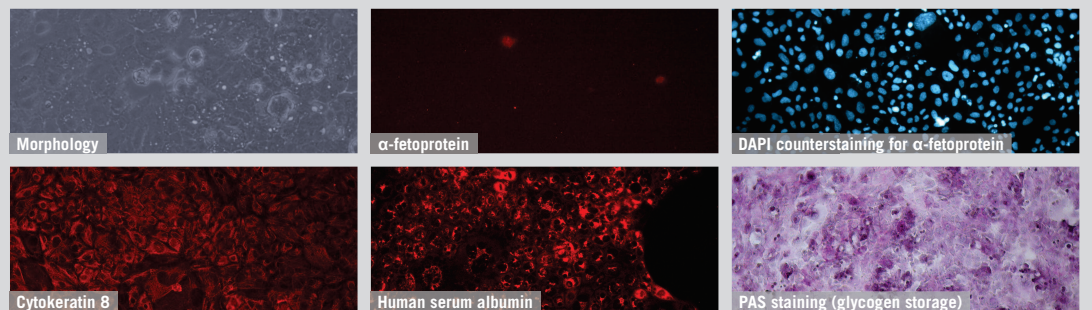
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Medicyte's role in the HeMiBio project is to provide an alternative cell source of hepatocytes and non-parenchymal liver cells for the development of a hepatic microfluidic bioreactor. For this purpose Medicyte uses its upcyte® technology consisting of a genetic manipulation step which causes differentiated primary cells to proliferate without inducing immortalization, uncontrolled cell growth, or significant loss of phenotype. After successful treatment, cells are expanded to a "working cell bank" and characterized and compared with the primary cells from which they were derived. Upcyte® cells are shipped to the project partners for co-culture and bioreactor optimization. In addition, bioreporters are introduced into upcyte® cells using Zinc Finger Nuclease-technology.



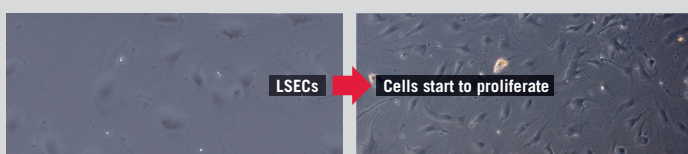
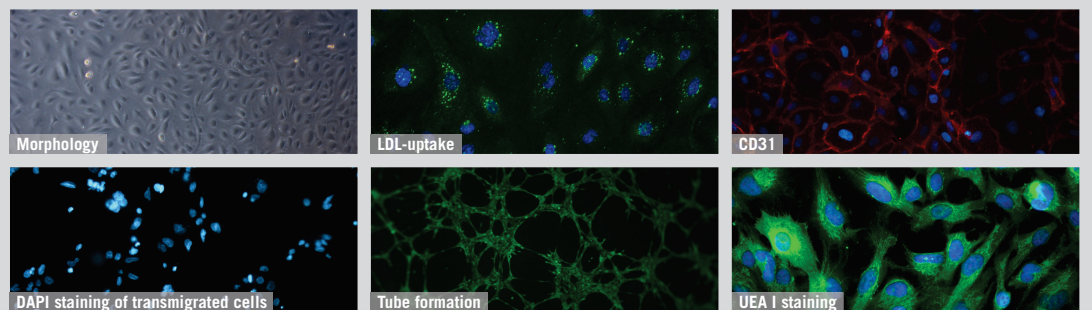
→ UPCYTE® HEPATOCYTES

Upcyte® hepatocytes express all the important markers. They exhibit adult cell morphology, express liver specific differentiation markers, and produce glycogen and human serum albumin. Furthermore, these cells exhibit measurable phase I and II metabolism and express a number of drug transporters. These cells have been used to differentiate between prototypical hepatotoxic and non-hepatotoxic compounds and are also applicable to genotoxicity assays, such as the micronucleus (MN) test.



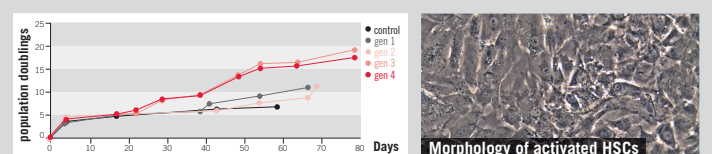
→ UPCYTE® MICROVASCULAR ENDOTHELIAL CELLS (mvEC)

As proof of principle of the upcyte procedure of endothelial cells we have successfully applied this technology to microvascular endothelial cells (mvEC). We were able to produce large batches of upcyte® mvEC such that they retained their specific endothelial characteristics, e.g. von-Willebrandt-Factor (vWF), CD31 and eNOS, expression as well as their biological functions, demonstrated by their ability to migrate, form tubules and take up LDH.



→ LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSEC)

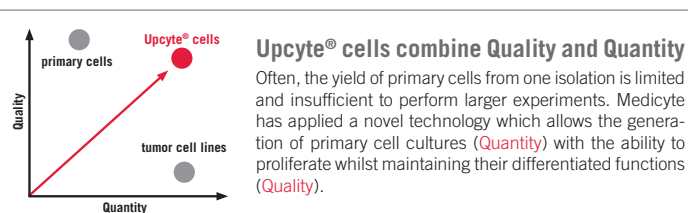
LSECs are difficult to isolate in pure cultures and low cell numbers are recovered. Upcyte® technology is currently being applied to produce these cells in larger quantities.



→ HEPATIC STELLATE CELLS (HSC)

Upcyte® gene combinations have been identified that result in an extended proliferation of transduced HSC. However, cells we used had already undergone activation. Therefore our aims are to upcyte® the cells before activation or to return upcyte® HSC to the quiescent state using activation inhibitors.

Morphology of activated HSCs



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SUMMARY

So far Medicyte successfully upcyted hepatocytes and mvECs. Upcyte® hepatocytes are available from several donors with different characteristics and have been provided to HeMiBio partners. Upcyte® mvECs are under investigation at present in co-culture experiments and in comparative studies to determine whether they can be used as an alternative to LSECs. Upcyte® technology is currently applied to LSECs and quiescent HSCs to produce these cells in larger batches.