A Co-culture System for a 3-D Human Liver Reconstruction on Chitosan Membranes
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Introduction
For the in vitro realization of an organ typical model a co-culture system constituted by hepatocytes and endothelial cells in chitosan membrane systems was evaluated. Chitosan, a mucopolysaccharide derived from chitin by deacetylation, is a biocompatible and biodegradable material and has been shown to be a well-defined matrix for hepatocyte attachment [1]. It’s well known that cell-cell communication between multiple cell types in vivo is essential to maintain differentiated cell functions. A direct contact with non parenchymal cells is required for longer maintenance of hepatocyte functions in vitro [2]. The aim of this study was the 3-dimensional liver reconstruction using a layered co-culture system of human hepatocytes and human umbilical vein endothelial cells (HUVECs) on chitosan membranes in flat and hollow fiber configuration.

Materials and Methods
Chitosan membranes were prepared in flat and hollow fiber configurations by phase inversion technique. After the preparation membranes were characterized in order to evaluate their morphological and physico-chemical properties [3-4]. Two different layered co-culture of human hepatocytes and HUVECs were obtained: i) HUVECs with 4 to 5 population-doubling levels were plated on membranes at a cell density of 15x10³ cell/cm², after 24h human hepatocytes were overlayed at a cell density of 150x10³ cell/cm²; ii) human hepatocytes were plated at a cell density of 150x10³ cell/cm², after 4h HUVECs were overlayed at a cell density of 15x10³ cell/cm². The morphological behaviour of cells was observed by light-inverted microscopy, by scanning electron microscopy (SEM) and by laser scanning confocal microscopy (LSCM). Liver specific functions were evaluated in terms of albumin synthesis, urea production and diazepam biotransformation.

Results
After 3 days a similar morphological behavior was observed in the two different co-culture systems with self assembly of tissues and heterotypic cell-cell interactions. A homogeneous distribution of cells in tight contacts was observed with the endothelial cells elongating and enclosing lumen-like structures with closely associated hepatocytes, very similar to the liver sinusoid. A similar morphological behaviour was observed on chitosan membrane as well as on collagen gel. On hollow fiber membrane the 3-dimensional distribution and tissue re-organization of cells was improved. The differentiated shape of hepatocytes in the co-culture systems was maintained for all the culture time as well as the differentiated liver specific functions.

Discussion and Conclusions
The strategy to co-culture hepatocytes with HUVECs on chitosan membranes in flat and hollow fiber configuration allows to generate tissue-like structures with a 3-dimensional morpho-architecture very similar to those observed in vivo. Human hepatocytes were cultured in microenvironment in which adhesion was improved by the materials and by cell communications with non parenchymal cells. The sinusoid-like structures were re-organized and the synthesis of extra-cellular matrix proteins were regulated. This engineered construct provides an interesting approach for manipulating liver tissue function in vitro and may be a valid tool for therapeutic and drug-screening application.

References