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Human hepatocyte functions in a crossed hollow fiber membrane bioreactor

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ABSTRACT

An important challenge in liver tissue engineering is the development of bioartificial systems that are able to favour the liver reconstruction and to modulate liver cell behaviour.
A crossed hollow fiber membrane bioreactor was developed to support the long-term maintenance and differentiation of human hepatocytes. The bioreactor consists of two types of hollow fiber (HF) membranes with different molecular weight cut-off (MWCO) and physico-chemical properties cross-assembled in alternating manner: modified polyetheretherketone (PEEK-WC) and polyethersulfone (PES), used for the medium inflow and outflow, respectively. The combination of these two fiber set produces an extracapillary network for the adhesion of cells and a high mass exchange through the cross-flow of culture medium. The transport of liver specific products such as albumin and urea together with the transport of drug such as diazepam was modelled and compared with the experimental metabolic data. The theoretical metabolite concentration differed 7.5% for albumin and 5% for urea with respect to experimental data. The optimised perfusion conditions of the bioreactor allowed the maintenance of liver functions in terms of urea synthesis, albumin secretion and diazepam biotransformation up to 18 days of culture. In particular the good performance of the bioreactor was confirmed by the high rate of urea synthesis (28.7 μg/h 10^6 cells) and diazepam biotransformation. In the bioreactor human hepatocytes expressed at high levels the individual cytochrome P450 isoenzymes involved in the diazepam metabolism. The results demonstrated that crossed HF membrane bioreactor is able to support the maintenance of primary human hepatocytes preserving their liver specific functions for all investigated period. This device may be a potential tool in the liver tissue engineering for drug metabolism/toxicity testing and study of disease pathogenesis alternatively to animal experimentation.

1. Introduction

Liver tissue constructs consisting of functional cells and artificial materials are being greatly studied for their applications in the clinical field for organ replacement and in the in vitro studies for drug development and metabolic diseases. The impact will be increasing for the coming decade in the design of in vitro physiological models to study disease pathogenesis and in the development of molecular therapeutics alternatively to animal experimentation. Animal models suffer from serious shortcomings regarding the prediction for a human situation as significant species differences in enzyme expression exist between man and animals. Isolated hepatocytes represent a good model of liver metabolism because they are able to perform the full range of known in vivo biotransformation, synthetic and detoxification functions [1,2]. However, hepatocytes rapidly lose their liver specific functions when maintained under standard in vitro culture conditions. In fact, static culture methods are characterized by an unstirred medium layer overlying cells attached to a gas impermeable substratum and are exposed to changes of nutrient concentration and catabolite accumulation on time. For liver cells, which are highly perfused in vivo, such conditions are susceptible to oxygen and nutrient limitations with consequent reduction of cell viability and functionality. A variety of culture methods have been developed to foster retention of hepatocyte functions including co-culture with nonparenchymal cells [3], culture in a sandwich collagen gel [4], synthetic extracellular

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matrix [5], in three-dimensional systems in spheroids [6,7] or scaffolds [8], and in a variety of dynamic systems such as bioreactors [9–13]. Bioreactors allow the culture of cells under tissue specific mechanical forces (e.g., pressure, shear stress and interstitial flow) augmenting the gas and nutrient exchange under complete fluid dynamics control that ensure the long-term maintenance of cell viability and functions [14,15]. Various bioreactor configurations have been explored for hepatocyte culture including membrane bioreactors (hollow fiber, flat, fiber network, spiral) [16–21] and by using several adhesive substrates [22–26]. Among the bioreactors hollow fiber membrane bioreactors meet the main requirements for cell culture: wide area for cell adhesion, oxygen and nutrient transfer, removal of catabolites and protection from shear stress [14,15]. Furthermore, hollow fiber membranes may serve as scaffolding material guiding the spatial organisation and microarchitecture of the liver tissue. Critical issues in the hollow fiber (HF) membrane bioreactors are the configuration of the bioreactor, the fluid dynamics and the membrane properties which depend on the cell adhesion and mass transport. Mass transfer across the membrane occurs by diffusion and/or convection in response to existing transmembrane concentration or pressure gradients. Both mechanisms of transport should be taken into account in the design of HF membrane bioreactors [14]. In the case of hepatocytes which are anchorage-dependent cells the membrane properties are critical not only for the transport but also for their interaction with cells. Surface properties favouring the cell functional and phenotypic maintenance [27–29] are required. Previously we have developed modified polyetheretherketone (PEEK-WC) membranes in flat configuration for hepatocyte culture [30]. This polymer owing to the presence of an isobenzofurane-1,3-dihydro-1-oxo- group in the polymer chain is soluble in common solvents and it can be used for preparing membranes with different properties by an inexpensive and flexible method [31]. Our studies demonstrated that PEEK-WC flat membranes are able to support the adhesion and metabolic functions of hepatocytes. PEEK-WC in hollow fiber (HF) configuration has been used also for lymphocytes culture [32].

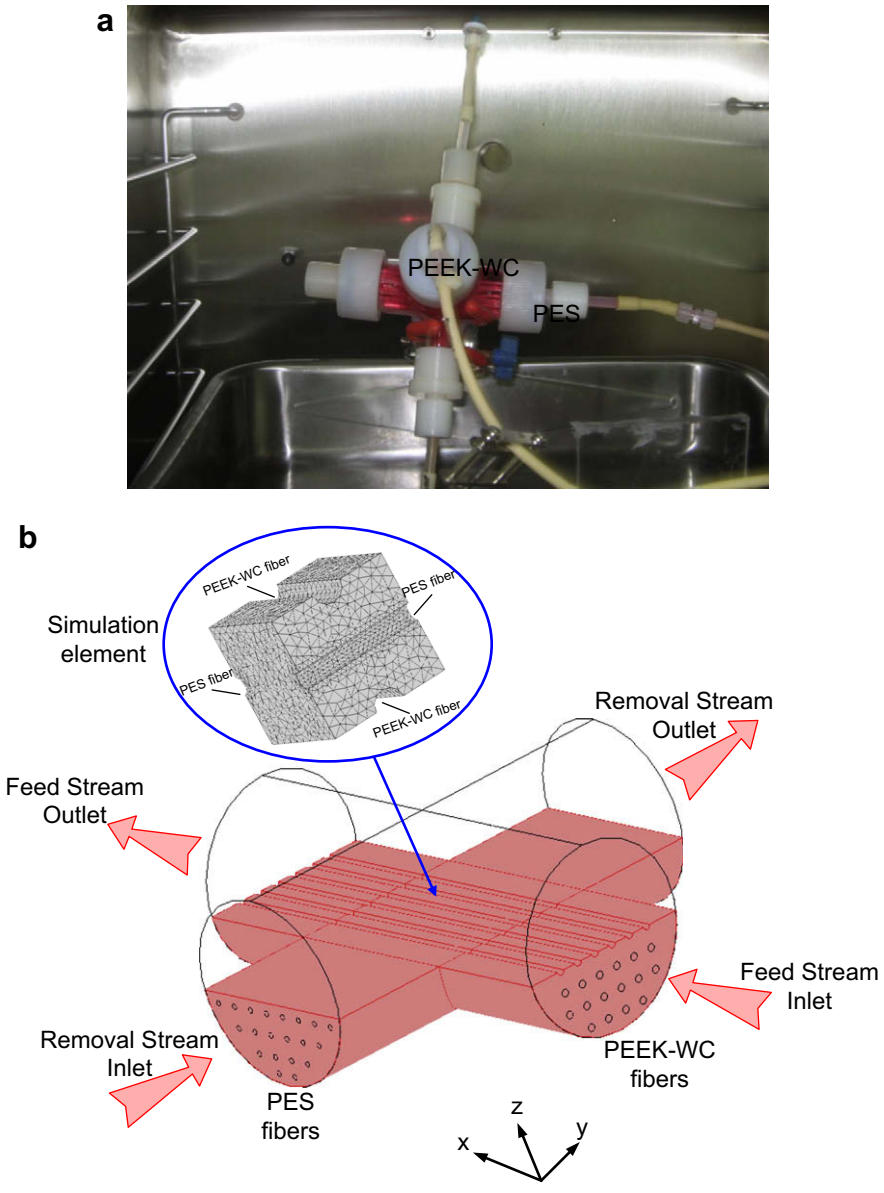

Fig. 1. Membrane bioreactor: (a) photograph. (b) Vertical section scheme of the crossed hollow fiber membrane bioreactor.