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Human lymphocytes cultured in 3-D bioreactors: Influence of configuration on metabolite transport and reactions

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ABSTRACT

Peripheral blood lymphocytes isolated from healthy human donors' buffy coat were cultured in membrane bio-reactors (MBR) designed in two different configurations: a conventional hollow-fiber (HF) bundle of modified polyetheretherketone (PEEK-WC) arranged in parallel, and a cross-assembled PEEK-WC and polyethersulfone (PES) HF membranes having different structural properties. Both bioreactors were experimentally compared in terms of metabolic activity of cultured cells, monitored over 8 days with respect to glucose uptake rate (GUR) and lactate production rate (LPR), and mathematically modelled by Computational Fluid Dynamics (CFD) method in order to investigate the impact of geometrical configuration and transport properties of biomaterials. The almost uniform trend of GUR from day 2 to day 7 (average of 0.0497 ± 0.0076 ng/h cell) and the low LPR (that decreased from an initial value of 2.92 ± 0.0055 pg/h cell to practically zero at day 8) provided evidence for superior performance of crossed-HFMBR in reproducing an optimal *in vitro* physiological environment with quite uniform concentration distribution of species in the extracellular space of the bioreactor and able to maintain lymphocyte viability and functions. The crossed HFMBR also resulted in an enhanced production of interleukin IL-2 over 8 days (average of 0.995 ± 0.25 pg/h/Mcell) and IL-10 in the first 3 days (average of 6.46 ± 0.28 pg/h/Mcell) which were up to one order of magnitude higher with respect to values measured in the parallel configuration.

1. Introduction

The expansion of T-lymphocytes has become of increasing importance in adoptive cell therapy for treatment of malignant diseases or viral infection as they exhibit a reduced toxicity and could provide a prolonged protection. The ex vivo expansion and transplantation of autologous antigen-specific T-lymphocytes obtained from resected specimens may represent an effective therapy for the treatment of metastatic melanoma, renal cancer, cytomegalovirus and Epstein–Barr virus induced malignancies [1]. Lymphocytes may be used also as biomarkers of target-organ susceptibility or as a marker of chemical effects and in the prediction of individual drug sensitivity alternatively to human liver biopsies [2,3].

The common procedure used for the expansion of lymphocytes are represented by static culture systems as multiwell plates, T-flasks, culture trays or culture bags [4–7]. These systems imply disadvantages that are related to oxygen and nutrients limitations and catabolite accumulation, low cell densities and uncontrolled process parameters. Dynamic bioreactors allow overcoming the limitations of traditional static batch systems offering perfusion of cells with nutrients and metabolites, high cell densities, implementation of control systems. Several devices have been explored for the *in vitro* culture of lymphocytes including hollow fiber bioreactor, stirred thank bioreactors and wave bioreactors [8–13] obtaining various results in cell growth. T lymphocytes have strict requirements and an optimal cell growth and viability can be achieved when cells are cultured in a well-controlled microenvironment that ensures an adequate mass transfer of nutrients and metabolites and removal of catabolites. Bioreactors using hollow fiber (HF) membranes with suitable molecular weight cut-off (MWCO) and adequate physico-chemical properties may meet these requirements. Critical issues in the design of the bioreactor regard the membrane properties and bioreactor configuration.

In this paper we compare HF membrane bioreactor (HFMBR) with different configuration for *in vitro* culturing of lymphocytes: parallel-fiber and crossed-fiber bioreactors. The

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parallel-fiber bioreactor (parallel-HFMBR) consists of modified polyetheretherketone (PEEK-WC) HF membranes, previously developed [13], which are assembled in a parallel manner and potted at each end in order to establish two separate compartments: an intraluminal compartment within the fibers in which the medium flows, and an extraluminal compartment or shell outside of the fibers where cells are cultured. The two compartments communicate through the pores in the fiber wall governing the transport of molecules. Hollow fibers provide a wide area for exchange of nutrients, metabolites and catabolites in a small volume and protection from shear stress. Cells grow around the fibers in the extracapillary space reaching high densities.

The crossed-fiber bioreactor (crossed-HFMBR) is based on the use of two types of HF membranes with different MWCO and physico-chemical properties cross-assembled in alternating manner: PEEK-WC and polyethersulfone (PES). The different HF membranes in the bioreactor perform also different functions: PEEK-WC HFs are devoted to provide the cells oxygenated medium containing nutrients and metabolites while PES HFs are devoted to remove from cell compartment catabolites and cell specific products. In this way the two HF membrane systems mimic the in vivo arterious and venous blood vessels.

Nutrient/catabolites mass transfer and reaction in the extracapillary space of the bioreactors where lymphocytes are cultured are evaluated and mathematically modelled in order to compare the parallel- and crossed-fibers configurations.

2. Materials and methods

2.1. Parallel-HFMBR

Parallel-HFMBR consists of modified polyetheretherketone (PEEK-WC) hollow fiber (HF) membranes assembled in parallel manner within glass housing (Fig. 1) [13]. The fibers are potted with polyurethane adhesive (Polaris Polymers, OH, USA) at each end in order to establish two separate compartments: an intraluminal compartment within the fiber, and an extraluminal compartment or shell outside of the fibers. The two compartments communicate through the pores in the fiber wall. The bioreactor (surface area: 128 cm^2, volume: 25 ml) was connected to the perfusion system consisting of a glass medium reservoir, oxygen-permeable tubing, a micro-peristaltic pump and a glass medium waste. The medium enters from the reservoir to the membrane bioreactor with a flow rate Q of 5 ml min^{-1} that was set on the basis of average retention time. Fresh medium was perfused in single-pass and the stream leaving the bioreactor Q_{out} was collected as waste until approaching the steady state. When the system reached the steady state, the stream leaving the bioreactor was recycled (Q_r) in order to obtain the accumulation of products.

2.2. Crossed-HFMBR

The bioreactor consists of crossed membrane system of 40 independent PEEK-WC HF and 40 polyethersulfone (PES) HF membranes used for the medium inflow and outflow, respectively [14] (Fig. 1). The two fiber systems were assembled in alternating manner and potted with polyurethane adhesive (Polaris Polymers, OH, USA) within glass housing. The fibers were potted at each end in order to establish three separate compartments: two intraluminal compartments within the PEEK-WC and PES fibers, and an extraluminal compartment or shell outside of the fibers. The bioreactor (volume: 35 ml) is connected to the perfusion circuit consisting of micro-peristaltic pump, gas-permeable silicone tubing, reservoir of medium and glass medium waste.

The oxygenated medium enters from the reservoir to the membrane bioreactor with a flow rate Q_f of 1.5 ml/min that was set on the basis of average retention time. Fresh medium was perfused in single-pass and the stream leaving the bioreactor Q_{out} was collected as waste until approaching the steady state. When the system reached the steady state, the stream leaving the bioreactor was recycled (Q_r) in order to obtain the accumulation of products.

2.3. PEEK-WC-HF membrane preparation

PEEK-WC HF membranes were prepared according to the well-known dry-wet spinning method. In order to prepare highly porous membranes, poly(-vinylpyrrolidone) (PVP K17 by BASF) was used as a pore forming additive. Membranes were prepared from solutions of PEEK-WC and PVP both at 15 wt.% in

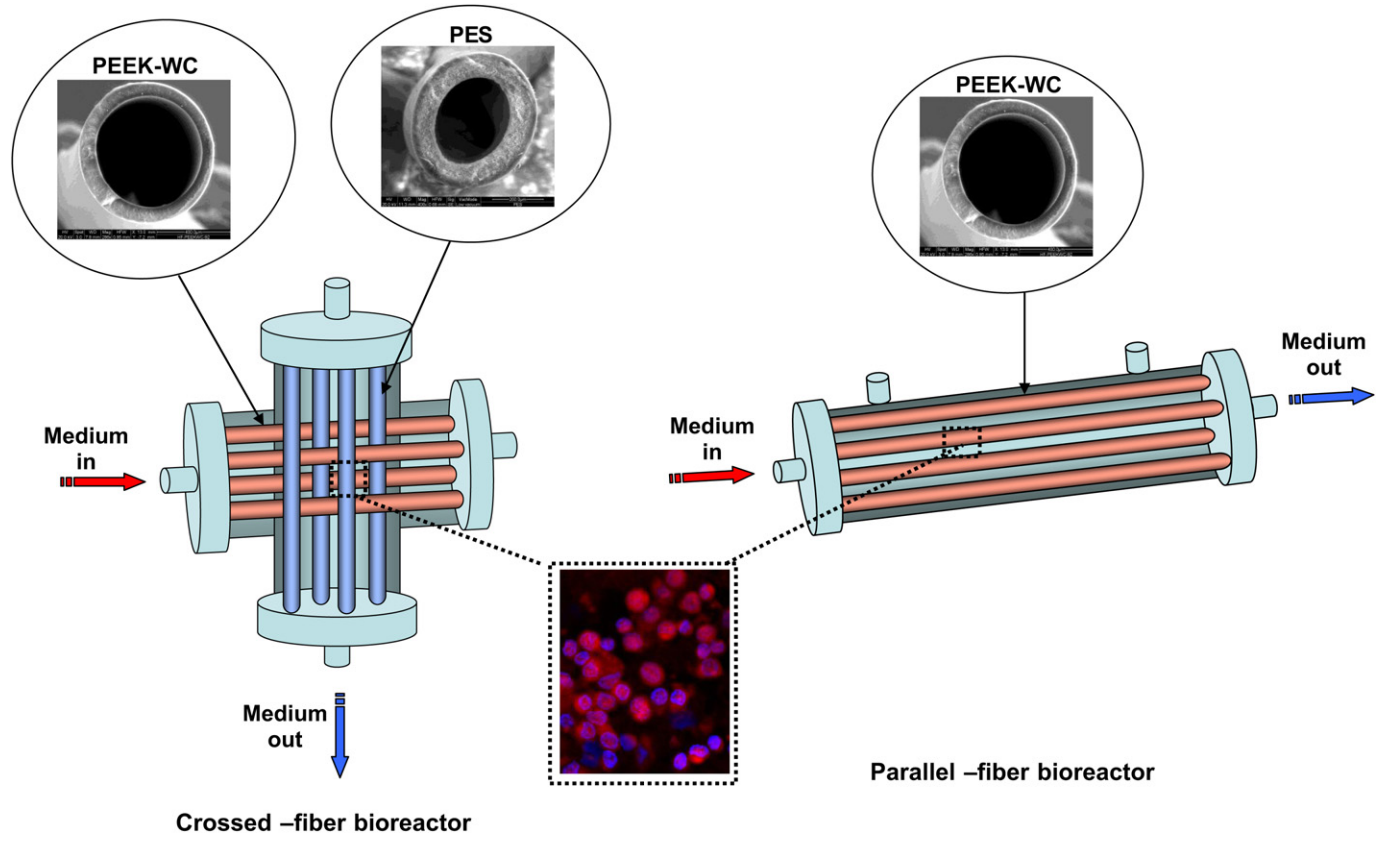

Fig. 1. Scheme of crossed fiber and parallel fiber bioreactors.