J. Biol. Res. 2002 n. 1 - Vol. LXXVIII - Rubbettino - Soveria Mannelli

Membranes in Bioartificial Organs

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Polymeric semipermeable membranes and membrane processes play a pivotal role in replacement therapy for acute and chronic organ failure and in the management of immunological disease. All clinical blood purification methods employ membrane devices [1]: for the intra and extra-corporeal treatment of patients with various pathologies for the removal of endogeneous or exogeneous toxins form blood (plasmapheresis, hemodialysis; hemo-diafiltration) or for the gas exchange with blood (blood oxygenation). The next generation of artificial organs and tissue therapies is almost certain to be similarly grounded in membrane technology.

Membranes of suitable molecular weight cut-off are used in bioartificial organs (e.g., pancreas, liver) using isolated cells, as selective barriers to prevent immune system components from getting into contact with the implant, while allowing nutrients and metabolites to permeate freely to and from cells. Currently two important areas of interest are the bioartificial pancreas for the treatment of insulin-dependent diabetes and the liver assist device for the temporary treatment of acute liver failure. Membrane capsules containing dopamine secreting cells also are being explored for treating of Parkinson's disease, a progressive brain disorder characterized by a deficiency of the neurotransmitter dopamine [2]. Immunoprotective membrane cell transplants are being investigated to treat other nervous system disorders. Polymeric membranes also are being explored to block cell adhesion or scar tissue formation, for example after surgery, and thus improve wound healing. In addition, the membranes are being investigated for prevention of restenosis (coronary artery narrowing) after angioplasty [3].

In membrane bioartificial organs, cells are compartmentalized by means of semipermeable membranes that permit the transport of nutrients and metabolites to cells and the transport of catabolites and specific metabolic products to blood. The membrane must avoid the contact between xenogenic cells and patient's blood to prevent immunological response and rejection of xenograft. Membranes act as means for cell oxygenation and in the case of anchorage-dependent cells as substrata for cell attachment and culture. As a result, the type of membrane to use in a bioartificial organ must be chosen on the basis of its permeability characteristics as well as on its physico-chemical properties related to the separation process.

The development of an extracorporeal liver assist device, using isolated liver cells could be a promising approach to support patients with hepatic failure until a liver transplant or a regeneration of liver partially injured.

Different devices have been proposed in literature. These devices differ from one other for the type of material used to construct the bioreactor: membrane, glass or non-woven fabrics; for configuration: flat or hollow fiber; for coating used: matrigel, collagen; for cell culture technique: microcarrier, spheroids, aggregates, etc, and for cell capacity.

Recently, we evaluated *in vitro* the performance of a full-scale flat membrane bioreactor (FMB) developed by Bader et al., that permits the culture of liver cells under *in vivo*-like conditions and at high-density culture [4-8]. In such bioreactor porcine hepatocytes are cultured within extracellular matrix between oxygen permeable flat-sheet membranes. Isolated liver cells are located at a distance of 10-20 μm of extracellular matrix. This bioreactor provides culture conditions that improve liver specific functions of liver cells in vitro. The FMB is able to provide an *in vivo*-like microenvironment for liver cells: hepatocytes are arranged as a plate in 3-D coculture with intermingled non-parenchymal cells. In contrast to other bioreactors the FMB is based on the organization of liver cells as a plate within extracellular matrix in which each individual hepatocyte has its own membrane support and thereby its own oxygen supply position. *In vitro* studies demonstrated that the performance of a scale-up FMB using porcine hepatocytes is stable over a period of about 3 weeks and compares well with that of other systems present in literature. Isolated hepatocytes cultured in the FMB reconstitute many of the features of the liver *in vivo*. The cell concentration inside of the FMB increased in the first days of culture and then remained constant until 18 days. Specific metabolic functions of hepatocytes in terms of albumin synthesis, ammonia elimination and urea synthesis are sustained for the investigated culture time demonstrating thus the long-term maintenance of functional integrity of hepatocytes cultured in the FMB.

In membrane bioartificial liver using isolated liver cells, semipermeable membranes play more functions: they act as immunoselective barriers, as means for cell oxygenation and provide a large area for cell attachment. All these functions are important for the maintenance of cell viability and specific functions. In our experimental study we

demonstrated that isolated rat liver cells cultured on oxygen-permeable membranes reconstitute many feature of the *in vivo* [9]. Cells cultured on polythetrafluoroethylene oxygen-permeable membranes maintained a morphological appearance of hepatocytes similar to their *in vivo* appearance and exhibited at high levels tissue specific functions *in vitro* in terms of albumin secretion, urea synthesis and drug biotransformation functions. This finding is of relevance for oxygen supply to cells *in vitro* both in batch and in large-scale bioreactor systems.

In a membrane bioartificial organ, cells come into contact with the membrane surface. Therefore, the response of the biological components depends on surface properties of the used membrane. Physico-chemical properties including surface composition, surface charge, surface energy, and surface morphology, may affect cell adhesion and behavior. Recently, studies performed on isolated hepatocytes cultured on semipermeable polymeric membranes have indicated that wettable and rougher surfaces enhance adhesion and metabolism of isolated hepatocytes [10-12]. In particular, measurements of the wettability of membrane, expressed by the contact angle in the presence of different liquids permitted to evaluate and to compare surface free energy components of membranes with different physico-chemical properties. Such measurements before and after modification of native membranes in culture medium resulted to be predictive index of their cytocompatibility and/or tissue biocompatibility. Therefore a material surface treatment might enable the adaptation of its surface free energy to biological requirements.

The adhesion and activity of liver cells are affected by hydrophilic properties of membranes: surface free energy and its components. We observed that cell adhesion increased with increasing base parameter of membrane surface tension. In particular on Cellulose Acetate (CA) and Polycarbonayte(PC) membranes hepatocytes formed large cellular aggregates while on hydrophobic membranes such as Polypropylene (PP) membranes cells spread to a large extent and the edge of cells were often indistinguishable in a network formed by fibrous proteins of extracellular matrix. For each membrane The base parameter of surface free energy affected the metabolic functions of liver cells. We found a correlation between urea synthesis and base parameter of membrane surface tension. Liver cells synthesise urea with a rate that increases with increasing of base parameter value of membrane surface tension. The metabolic activity is particularly expressed at high levels when cells are cultured on PC and CA membranes.

These results suggest that there is a marked effect of the physico-chemical properties of membranes on cell adhesion and functions. As a result, independently on the type of native polymeric membranes it is possible to improve cell adhesion and specific functions by changing their surface tension and their components. On the basis of our results, CA and PC membranes resulted to be good substrata for liver cell culture *in vitro* promoting cell adhesion and specific functions in term of urea synthesis.

To this purpose, researches on interactions of cells with novel membranes made in our laboratory are running in order to develop more cytocompatible and biocompatible membranes.

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