

Characterization of Membrane Materials and Membrane Coatings for Bioreactor Units of Bioartificial Kidneys

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Statement of Purpose: Kidney disease is a worldwide health problem, with increasing incidence, prevalence, high costs and poor outcomes. Bio-artificial kidneys (BAKs) provide a promising approach to address this problem as they could provide more comprehensive functions than conventional hemodialysis. BAKs contain renal cells grown on synthetic polymer membranes. For clinical applications, human primary renal proximal tubule cells (HPTCs) are the most relevant cell type. As membrane materials, commercially available hollow fiber membrane materials, such as polyethersulfone/polyvinylpyrrolidone (PES/PVP), polysulfone/polyvinylpyrrolidone (PSF/PVP) and regenerated cellulose (RC), were tested as they are used for hemodialysis. However, we found that they do not support HPTCs' growth and survival [1, 2]. To solve this problem, in this study, we have applied a variety of surface treatments and extracellular matrix coatings to PES/PVP and PSF/PVP membranes. HPTC performance on these membranes was evaluated so that an appropriate membrane material and/or membrane coating could be selected for bioreactor units of BAKs.

Methods PES/PVP and PSF/PVP flat membranes were prepared by phase inversion methods. The membrane materials were treated with poly (maleic anhydride-alt-1-octadecene) (PA-18, MW ~ 30–50 kDa), poly-L-lysine (PLL, 0.01%), 3,4-dihydroxy-L-phenylalanine (DOPA), and hydrogen peroxide (31%). After surface treatments, the surface of these materials was characterized by water contact angle and zeta potential measurements and X-ray photoelectron spectroscopy (XPS). Extracellular matrix (ECM) proteins were used to coat the PES/PVP and poly (ethylene terephthalate) (PET, Transwell®) membranes. HPTCs (ATCC, Catalog Number: PCS-400-010, Rockville, MD, USA) were cultured in renal epithelial cell basal medium supplemented with 0.5% fetal bovine serum (FBS) and renal epithelial cell growth kit-BBE (ATCC). Only early passage cells were used in our experiments. Renal cell performance on these surface-treated or ECM-coated membranes was evaluated by measuring cell attachment and cell proliferation. Indirect immunostaining of the tight junction protein zonula occludens (ZO)-1 was used as an indicator of epithelial differentiation.

Results: In order to investigate whether ECM coatings could sufficiently improve HPTC performance on membrane materials used for hemodialysis, we analyzed cell growth on uncoated or ECM-coated PES/PVP membranes. Uncoated and ECM-coated PET Transwell® membranes were used as the control. Cell proliferation on PES/PVP and PET membranes was determined by cell counting. The results showed that HPTC performance was

much more influenced by the underlying membrane material than by the ECM coating. Cell numbers always remained very low on PES/PVP membranes, for both the uncoated and the coated membranes. As sufficient improvements could not be achieved by applying ECM coatings, surface modifications of the conventional membrane materials were then used. Next, we tested how the different treatments and changes in surface properties affected human proximal tubule cell attachment. As the best results in terms of cell attachment and proliferation were obtained with DOPA-coated membranes, we further investigated the formation of differentiated epithelia by HPTCs on DOPA-coated PES/PVP. ZO-1 immunostaining patterns indicated that HPTCs did not form properly differentiated epithelia on DOPA-coated PES/PVP membrane. As such, we have applied both DOPA and collagen IV coating on PES/PVP and PSF/PVP membranes. HPTC performance was markedly improved as shown in Figure 1.

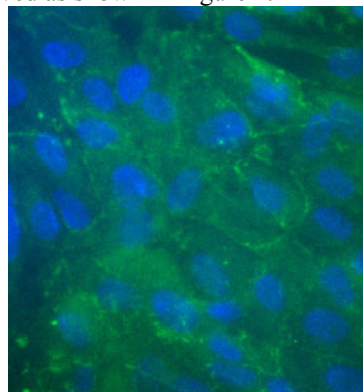


Figure 1. HPTC performance on double-coated PES/PVP membrane. (ZO-1: green, DAPI: blue)

Conclusions: Our results showed that the materials used in BAKs thus far would not be suitable for applications with HPTCs. Single ECM coating is insufficient to improve HPTC performance on PES/PVP polymeric membranes. Differentiated epithelia were successfully formed by HPTCs on PES/PVP or PSF/PVP membranes with combinations of DOPA and ECM coatings. The results suggest that the double coating procedure can be applied to a wide variety of commercial membrane materials to improve their cytocompatibility for applications in bioartificial organs.

References:

1. Ni M, Teo JCM, Ibrahim MSB, Zhang K, Tasnim F, Chow P, Zink D, Ying JY. *Biomaterials*, in press.
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