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Partner in Research

Heterogeneous surface modification of hollow fiber membranes and the utilization of affinity membranes in purification processes

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Introduction

Hollow fiber micro-bioreactors are used in different fields of application in biotechnology processes, e.g. classical filtration processes, cultivation of different test organisms, immobilization of enzymes as biocatalysts (enzyme micro-bioreactor) and product purification. The purification of target molecules (metabolites, proteins) can be achieved by using convenient modified hollow fiber membranes. For high-throughput experiments an arrangement of several micro-bioreactors in parallel can be realized. Furthermore, a combination of cultivation technology and following downstream processes (integrated downstreaming) offers a great potential in microbiotechnology. The miniaturization of the reactor system as well as the integration of operation steps positively influence the time- and cost-intensive downstream process.
The broad application of hollow fiber micro-bioreactors in various areas of biotechnology is still restricted due to a limited functionality of the membranes. With this work a straight forward procedure for membrane functionalization is presented.

Hollow fiber membranes

Three different commercially available hollow fiber membranes which consist of polyethersulfone (PES) or polysulfone (PS) are used in modification procedure. Informations concerning the pore size or molecular weight cutoff (MWCO) and inner/outer diameter are shown in Table 1.

Table 1: Specifications for used PES and PS hollow fiber membranes.

Hollow fiber membrane	Pore size / MWCO	Inner / Outer diameter [m]
MicroPES	0,2 m	300 / 500
Low Flux PS	5 kDa	200 / 280
High Flux PS	30 kDa	200 / 280

PES and PS are suitable membrane polymers for many biotechnological applications due to their resistance to thermal and biological degradation. Such membranes are usually preferred for their good chemical resistance, low fouling characteristics and broad pH range (pH 2-12).
The main disadvantages of these polymers are their undesirable surface properties. The membrane surface is hydrophobic and inert which means the lack of convenient functional groups.

Modification strategy

In the first step of the modification sequence, PES/PS hollow fibers were modified by reacting terminal hydroxy groups with diepoxide ethylene glycol diglycidyl ether (EGDGE). For increasing loading capacity hydroxyethyl cellulose (HEC) polymers were bound to the epoxy groups (step 2). In the third step, second epoxidation produced final polymers containing reactive epoxy groups on the hollow fiber surface (shown in Figure 1).

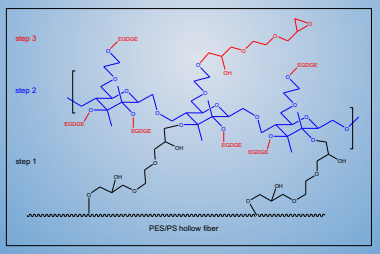

Figure 1: Stepwise modification of PES/PS hollow fiber hydroxy end groups with EGDGE, followed by coupling of HEC and second EGDGE epoxidation.

Loading capacities

Table 2 shows a binding capacity between 0.44 and 0.56 µmol of EGDGE / g hollow fiber in relation to used fiber material in the first reaction step. After linking HEC (step 2) to hollow fiber surface and second epoxidation (step 3) the concentration of bound epoxy end groups significantly increases. Loading capacities between 7.03 and 9.88 µmol epoxide / g fiber were achieved. The reaction sequence illustrates how functionality of these hydrophobic fibers can be amplified. In comparison to first epoxidation the epoxide concentrations after amplification increase by 12-22 fold. Amplification of reactive hydroxy end groups is based on covalently linked HEC.

Table 2: Loading capacities of bound epoxide groups and HEC polymers.

Hollow fiber material	Loading capacity / g hollow fiber			
	Step 1	Step 2		Step 3
	mol Epoxide	mol Epoxide	mg HEC	mol Epoxide
MicroPES	0.46 ± 0.09	0.09 ± 0.13	11.8 ± 2.3	8.59 ± 1.15
Low Flux PS	0.44 ± 0.07	0.19 ± 0.19	11.2 ± 2.3	9.88 ± 0.93
High Flux PS	0.56 ± 0.07	0.23 ± 0.19	9.9 ± 2.3	7.03 ± 0.92

SEM analyses of functionalized MicroPES membranes

It is important that the functionalization of hollow fibers does not damage the membrane surface. To assure intact hollow fiber surface scanning electron microscopy analyses (SEM) were performed. All SEM images were made with a magnification of 5000-fold.
In comparison to the untreated MicroPES membrane (A) the pore structure of functionalized membranes (B, C) is not negatively influenced by treating the membrane with HEC and EGDGE, respectively. Thus, the homogeneity of pore structure can be retained and functionalized membranes can be used for further modification reactions.

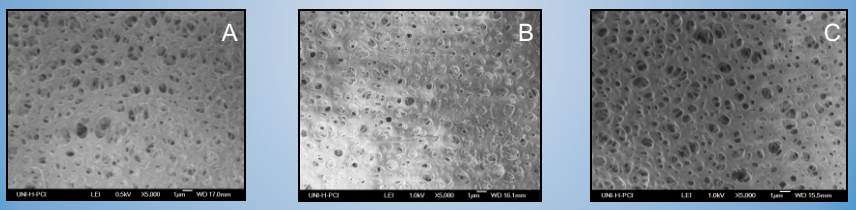

Figure 2: SEM analyses of MicroPES flat-sheet membranes. A = untreated, B = after coupling of HEC (step 2), C = after second epoxidation (step 3). Field-Emission Scanning Electron Microscope JEOL JSM-6700F.

Final coupling reactions

From the epoxidized PES hollow fiber membrane, a wide variety of bifunctional amine derivatives can be bound to the epoxy groups.
Modified fibers with terminal hydroxy or rather amine groups varying in spacer length (blue columns) and terminal dihydrazide and carboxy groups (yellow columns) can be produced. Furthermore, it is possible to generate strong and weak cation and anion exchange membranes (red columns). The last coupling reaction is refered to the immobilization of IDA for producing hollow fibers with chelating properties (green column).

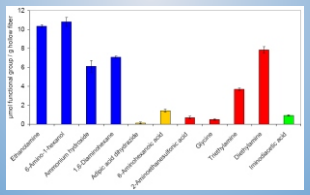

Figure 3: Loading capacities of final coupling of different bifunctional amine derivatives to epoxidized MicroPES hollow fibers.

Adsorption of metal ions and model amino acid histidine

The chelating IDA-membranes were complexed with different divalent metal ions. Immobilized metal ion affinity PES hollow fiber membranes were used for adsorption of histidine. Determination of bound and unbound histidines was performed by HPLC analysis.
With the metals tested, the loading capacity decreases in the following order: Co > Cu > Ni. Histidine loading capacities were determined by differentiation measurement.

Table 3: Metal-bonding capacities of affinity MicroPES hollow fiber membranes and their sorption capability for histidine.

Hollow fiber membrane	Loading capacity / g MicroPES hollow fiber	
	Metal-bonding mol Me	Differentiation values [a] mol His
MicroPES-IDA-Cu	8.78	8.53
MicroPES-IDA-Ni	8.15	6.30
MicroPES-IDA-Co	12.68	11.01
MicroPES-IDA-Zn	----[b]	4.92

[a] Differentiation between histidine concentrations of loading solution and supernatant
[b] Not determined

Summary

This work shows the possibility to functionalize commercially available PES and PS hollow fiber membranes with different reactive ligands. Starting from a limited number of hydroxy groups on hollow fiber surface it was possible to convert these hydrophobic membranes into hydrophilic ones.
SEM analyses demonstrate that the developed modification protocol assures intact hollow fiber surface. On the basis of epoxidized fibers the reactive epoxy ring in the membrane is able to undergo further reactions by nucleophilic substitution. A wide variety of affinity membranes with different terminal groups can be created.
Especially ion exchange and IMAC hollow fiber membranes can be used in downstream processes. Here, concentrating of dilute histidine solution was investigated by application of IMAC hollow fibers.

This Project is granted by DBU