

Supporting Information

A strategy to surface-graft stimuli-responsive hydrogels using scCO₂

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1. Materials

Cytopore 2 (CYT2) and cytodex (CYT3) microcarriers were purchased from GE Healthcare. Polysulfone (PS), $M_w = 67,000$) in pellet form, *N*-isopropylacrylamide (NIPAAm, purity $\geq 97\%$), *N,N*-dimethylformamide (DMF, purity $> 99\%$) and bovine serum albumin (BSA) (MW = 66 kDa, purity $\geq 98\%$) were purchased from Sigma-Aldrich. *N,N*-diethylacrylamide (DEAAm) was purchased from Polysciences. Polyacrylonitrile and native PS/PAN (90:10) membranes were synthesized and prepared in our laboratory following the procedure described in detail by Barroso *et al.*^{1,2} Carbon dioxide (CO₂) was supplied by Air Liquide with 99.998 % purity.

2. Methods

2.1. *Surface modification of polymeric structures*

Microcarriers CYT2 and CYT3 and PS/PAN membrane (Fig. S1 A) were activated by Argon plasma treatment for further coating with PNIPAAm and PDEAAm, respectively, in supercritical critical carbon dioxide. The plasma treatment was carried out in a radio frequency plasma reactor (Plasma system FEMTO, version 5). Polymeric structures were introduced in the plasma chamber which was thoroughly purged with a continuous flow of argon to reduce trace amounts of air and moisture. During the treatment, the argon flow was adjusted in order to keep a constant pressure of 0.3 Torr inside the chamber. A power of 60 W was applied during 3 minutes. At the end of the experiment the plasma chamber was ventilated (Fig. S1 B) and the samples were immediately introduced in the high pressure cell for further coating. In coating procedures activated microcarriers and PS/PAN membrane were placed inside the high pressure cell shown in Fig. S1 C and scCO₂ was used as carrier of the monomer into the activated polymeric structures. The interior of the high pressure cell is divided in two parts with a porous structure in order to avoid any contact of polymeric structures (in the superior part) with the monomer inferior part. In microcarriers coating it was used 80 mg of NIPAAm and for PS/PAN membrane coating it was used 1mL of DEAAm.

Coating procedures were performed at 37 °C and 20 MPa, during 24 hours under stirring. At the end of the coating procedure thermoresponsive structures were washed with fresh high-pressure CO₂ (37 °C, 20 MPa for one hour) in order to extract the remaining residues of unreacted monomer being already to be used.

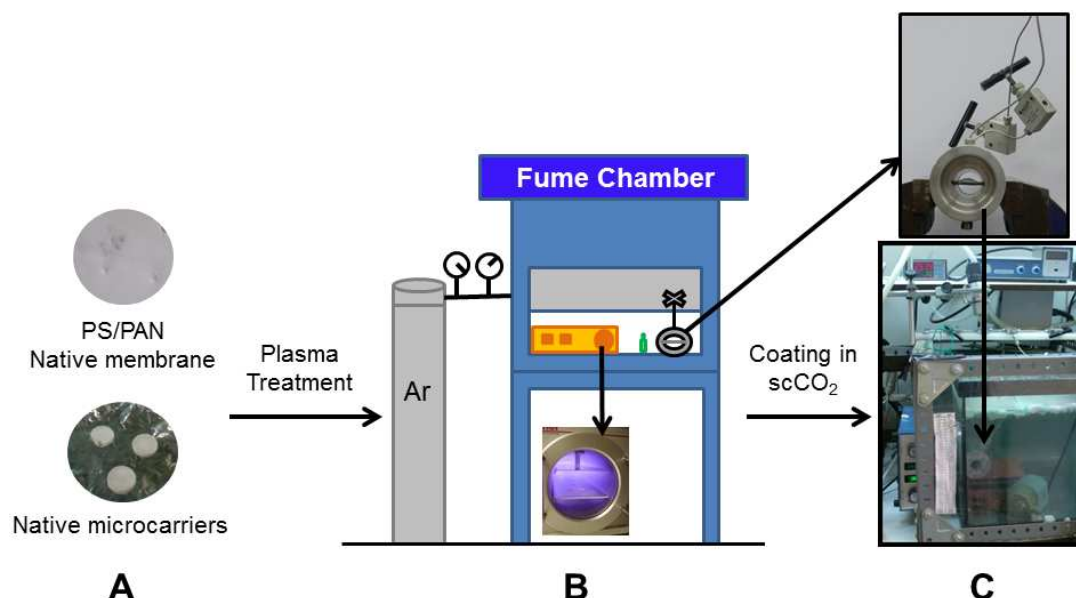


Fig. S1. (A) polymeric structures (B) polymeric structures activation by plasma treatment followed by high pressure cell preparation (introduction of activated polymeric structures in a high pressure cell with stirrer and monomer) for further polymerization in scCO₂ (C) typical high pressure cell for polymeric structures coating as well as real apparatus of polymeric structures coating.

2.2. *Characterization of polymeric devices*

Scanning electron microscopy (SEM) of the materials before and after coating was performed in a Hitachi S-2400 equipment, with an accelerating voltage set to 15 kV. All samples were coated with gold before analysis.

Membrane porosity and pore size distribution were determined by mercury porosimetry in a Micromeritics Auto Pore IV mercury porosimeter using sample weights between 0.080-

0.130 g in order to obtain a good distribution. These analyses were performed in two steps, first applying low pressure at 345 kPa and in the second applying high pressure at 223 MPa. Fig. S2 shows the average of medium pore size diameter variation of PS/PAN membrane before and after coating with PDEAAm. It is notorious the decrease of average of medium pore size diameter of PS/PAN membrane after PDEAAm coating which clearly justify the efficient PDEAAm coating using the integrated modification process herein presented.

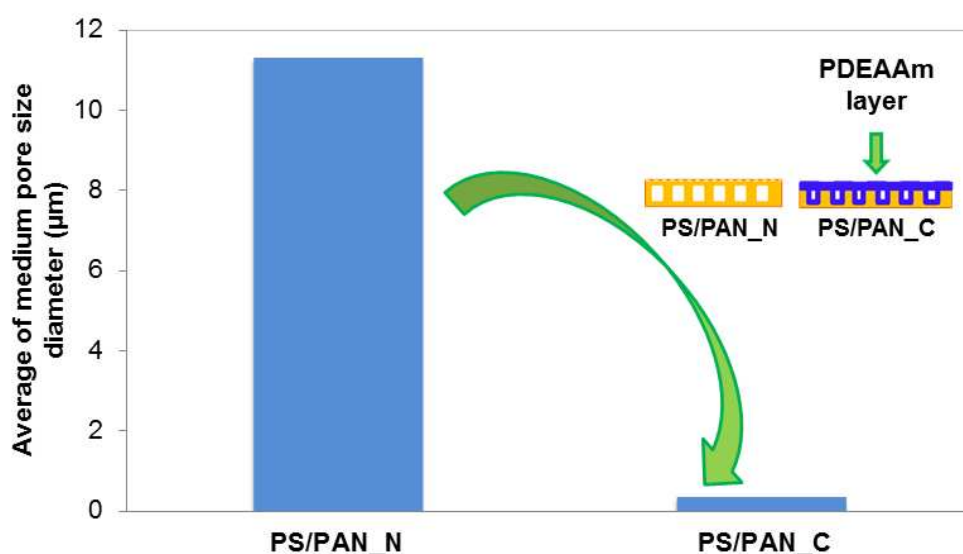


Fig. S2. Average pore size diameter of PS/PAN membrane before and after coating with PDEAAm.

The surface composition for the different devices was studied by X-Ray Photoelectron Spectroscopy (XPS) using the non-monochromatic Al K α radiation ($h\nu = 1486.6$ eV) from a Kratos Analytical XSAM800 equipment. Squared pieces of each membrane were held to the sample holder using a spring. Details about operation parameters and data treatment can be found in the literature.³ No flood gun was used for charge accumulation compensation. For charge shift correction purposes, two different references were used: for polysulfone/polyacrylonitrile (PS/PAN) sample, since the main polymer in the blend is the polysulfone, sp² carbon was used at binding energy, BE = 284.7 eV;⁴ for the CYT2 and CYT3 samples, both before and after covering, as well as for the covered membrane, the -CH₂- C 1s

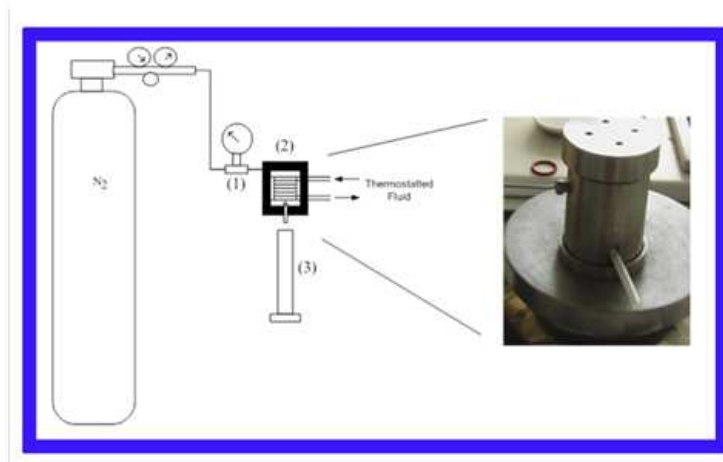
BE = 285 eV was used.⁴ For quantification purposes, sensitivity factors were: C 1s: 0.25; O 1s: 0.66; N 1s: 0.42; S 2p:0.54.

The swelling tests were performed using different approaches according to the polymeric structures. In order to determine membranes water uptake ability dynamic swelling and shrinking were followed during one week using the procedure reported previously.²

In case of microcarriers, their thermoresponsive behaviour was evaluated with an automatic Malvern Morphology G3 optical microscope (Malvern Inc, UK) kept inside a temperature-controlled chamber with an infrared lamp coupled to a PID temperature controlled. All PNIPAAm coated microcarriers were dispersed in PBS pH 7.4 and culture medium at 25 and 37 °C.

The tensile properties of the membranes were determined with tensile testing equipment (MINIMAT firm-ware v.3.1) at room temperature.²

The procedures for water permeability measurement and BSA filtration are described in detail in previous work.² Fig. S3 shows the apparatus for water flux measurements and protein filtration (A) and (B) Permeation, recovery and retention values for PS/PAN membrane coated with PDEAAm at different temperatures, 25 and 37 °C. Data from Fig. S3 B, show that higher BSA permeation values were obtained for 37 °C due the hydrophobic behaviour of PDEAAm. At 37 °C PDEAAm chains are hydrophobic, the hydrogen bonds are broken and consequently the polymer collapse opening the surface pores for protein permeation (90%). High permeation values led to low recovery values (10% at 37 °C). At 25 °C the polymer chains are highly hydrated establishing hydrogen bonds with water. Consequently, these interactions drive to an expansion of polymer chains that block the pores limiting the protein permeation (43%). Thus, low permeation values led to high recovery values, specifically, 57 % at 25 °C. However, as the retention values were below the experimental limit of detection for both temperatures, it means that no measurable quantities of BSA were retained on the membrane and consequently no fouling behaviour was registered.

A**B**

	Permeation		Recovery		Retention	
	(%) ^a		(%) ^b		(%) ^c	
	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
PS/PAN_C	43	90	57	10	0	0

^a Related with the amount of BSA that pass through the membrane during the loading step.

^b Related with the amount of BSA which is possible to elute in washing step.

^c Related with the BSA of protein which was impossible to recover from membrane.

Fig. S3. (A) Real filtration apparatus: (1) manometer, (2) high pressure cell filtration, (3) collecting reservoir and (B) BSA permeated, recovered and retained using PS/PAN membrane coated with PDEAAm at different temperatures, 25 and 37 °C.

References

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