Polyvinylpyridinium-type gradient porous membranes: synthesis, actuation and intrinsic cell growth inhibition†

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Gradient porous membranes were prepared from a poly(4-vinyl-pyridinium)-based poly(ionic liquid) together with carboxylic multi-acid compounds *via* electrostatic complexation. Their unique tuneable actuation kinetics in a humid environment as well as their intrinsic cell toxicity were studied.

Polymer actuators are capable of transforming changes occurring on a molecular level inside the material to responses detectable on a macro-scale, *i.e.* bending, swelling/deswelling, solubility changes, and alteration of surface characteristics, to name a few.[1–5] These changes may be induced by a variety of material-specific stimuli, which include pH, temperature, ionic strength, or electric and magnetic fields.[6] In this regard, polyelectrolyte membrane actuators have especially attracted much attention due to their high charge density and their potential applications in fields such as energy conversion, catalysis and electro-active sensors.[2,3,7–13] Stable polyelectrolyte membranes are usually prepared by the layer-by-layer (LbL) technique.[14–20] To fabricate high performance polyelectrolyte membrane actuators with fast kinetics and high sensitivity, a possible design is to make these films highly porous to accelerate the mass diffusion and to amplify the interfacial interaction.[21] Synthetically this task is addressable *via* LbL on porous templates, which yet delivers products of very limited amount.

Our group has previously reported the synthesis of a hierarchically structured porous poly(ionic liquid) (PIL) membrane by electrostatic complexation of a hydrophobic imidazolium based PIL with poly(acrylic acid) (PAA).[22] Here PILs, the polymerization products of ionic liquids,[23–28] were employed

as innovative polyelectrolytes that facilitate the pore generation on account of their ionic, but at the same time water-insoluble features.[5,24,29–33] The role of PAA was to neutralize the polycation and to electrostatically cross-link the PIL porous network under basic conditions. Expanding this work, our group has created an ultra-fast porous polymer membrane actuator responding to solvent vapour with unprecedented bending speed and stability.[21] The membrane actuation stems from a gradient in the degree of electrostatic complexation (DEC) of the PIL with a multivalent acid along the membrane cross-section. Applying the same principle of gradient complexation, we have been able to tune the pore size of these membranes from the nm to μm range using a series of benzoic acids of different multivalency.[34] From a chemical structure standpoint, these porous membranes and actuators have so far been only polyvinylimidazolium based. It is therefore of scientific curiosity to explore alternative polymers as building blocks to expand the synthetic scope of porous PIL membrane actuators.

In this communication, we applied the membrane fabrication principle to a poly(4-vinyl pyridine) (P4VP) derived PIL, which together with various types of multivalent organic acids builds up porous gradient polyelectrolyte membranes. Scheme 1 illustrates the chemical structure of the multi-acid derivatives used in this work. These membranes feature a tuneable bending speed upon change from a wet to a dry environment, and are of intrinsic cell toxicity, making them potentially applicable as actuators with antimicrobial or antifouling properties.

The PIL, poly[1-cyanomethyl-4-vinylpyridinium bis(trifluoromethane sulfonyl)imide] (PCMVPy-Tf$_2$N), was synthesized by post-modification of commercially available P4VP ($M_w \sim$ 60 kDa) rather than by the traditional route of polymerizing an ionic liquid monomer. In detail, P4VP was reacted with excessive bromoacetonitrile, followed by anion exchange to replace Br$^-$ by bis(trifluoromethane sulfonyl)imide (Tf$_2$N$^-$) (Scheme 1a).[35] A quantitative quaternization was confirmed by proton nuclear magnetic resonance. Following our previous reports,[21,22,34] the polymer membrane was synthesized by ionic complexation of the cationic PIL with *in situ* deproto-

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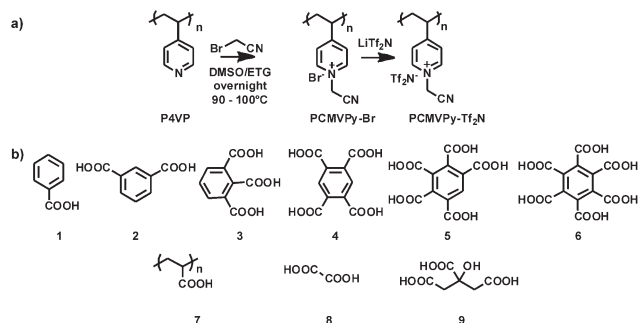[b]*Max Planck Institute of Colloids and Interfaces, Dep. of. Biomolecular Systems, Arnimallee 22, 14195 Berlin, Germany*
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a)

P4VP   PCMVPy-Br   PCMVPy-Tf$_2$N

b)

1   2   3   4   5   6

7   8   9

Scheme 1 a) Synthesis of polyvinylpyridinium-type PIL, PCMVPy-Tf$_2$N, by post-modification from poly(4-vinyl pyridine) (P4VP). (b) Structures of the acids used for electrostatic complexation. ETG denotes ethylene glycol.

Table 1 Average pore sizes of the membrane cross-section of PCMVPy-Tf$_2$N with different acids employed as cross-linkers

Acid	1	2	3	4	5	6	7	8	9
Pore size/nm	—	460	270	155	174	100	300	216	70

nated organic multi-acid derivatives (Scheme S1†). In a typical run, the PIL and the acid were dissolved at a molecular level in dimethylsulfoxide (DMSO), cast onto a glass plate and dried at 80 °C in air for 2 h. This non-porous dry polymer film was then immersed into aqueous NH$_3$ (0.2 wt%) in order to induce pore formation and simultaneous PIL-acid complexation. As NH$_3$ is gradually penetrating into the polymer film from the top (side facing NH$_3$ solution) to the bottom (side facing the glass slide), the resulting product shows a gradient in its DEC, being highest on the top and lowest at the bottom. The sulfur content along the membrane cross-section was taken as a quantitative measure of the DEC, as the Tf$_2$N$^-$ counteranions of the PILs, the only source of sulfur, are replaced by carboxylates in the step of electrostatic complexation. Monitoring therefore the sulfur content in the cross-sections of the membranes by energy dispersive X-ray diffraction (EDX) analysis, the gradient in the DEC was confirmed. The amount of sulfur is lowest in the membrane top, where the crosslinking density is the highest and increases gradually towards the bottom. Moreover, similar to previously reported polyvinylimidazolium counterparts, the membranes from PCMVPy-Tf$_2$N and the multivalent organic acids show a gradient in their pore size with the smallest pores in the denser upper part and larger pores in the less cross-linked bottom (Fig. 1b and c).[34]
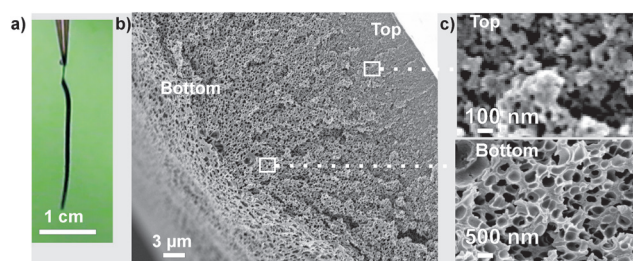
In order to show that PCMVPy-Tf$_2$N may be generally complexed by multivalent acids, polymeric and low molecular weight organic acids (2–9) were employed as cross-linkers, yielding membranes with average pore sizes ranging from 460 down to 70 nm. The average pore sizes of the membranes followed the same trend as that reported for polyvinylimidazolium membranes with benzoic acid derivatives,[34] *i.e.* acids of higher multivalency yield membranes with smaller pore sizes (Fig. S5,† Table 1 and Table S1†). The higher multivalency results in overall higher cross-linking density, which is important to stabilize smaller nanosized pores.

With these porous membranes in hand, we started to investigate their actuation properties. Generally speaking, membranes of PCMVP-Tf$_2$N and acids 2–9 are all responsive to water. Placed into a wet environment, the membranes have a stretched shape, whereas upon drying they bend into loops with the upper part of the membrane inward. Depending on the type of acid used for network formation, the bending degree and speed differ significantly. In a systematic study, benzoic acid derivatives 2–6 were employed as a model system, where only the multivalency of the benzoic acids varies. The humidity sensing properties of these membranes were studied by taking membrane stripes with dimensions of 30 mm × 2 mm from a water-filled petri dish, blotting the bulk water from the stripe surface by filter paper, and recording immediately the time-dependent bending angle (unit in rad) by using a digital camera. In order to obtain representative and comparable results, the bending angle was multiplied by the thickness of the membranes, which varied depending on the acid (Fig. 2a) and is known to have a major influence on the bending behavior of materials.

The maximal bending angle and the bending speed increase with the multivalency of the benzoic acid (Fig. 2a and b). The bending speed is lowest for membranes prepared from PCMVPy-Tf$_2$N and acid 2, reaching a maximal angle within 110 s (see ESI† for angle calculations). This membrane fails to recover its original shape after being placed back in water; that is, the humidity sensing property is irreversible. For membranes with acids of higher multivalency, the bending and unbending speeds in the dry and wet states increase significantly and the actuation becomes reversible (Fig. 2c). Membranes of acid 6 show the fastest kinetics, curving within 30 s to its maximal bending angle.

The water responsiveness of these membranes is traced back to the gradient in the DEC from the top to the bottom of the membranes. The dehydration of the charged polymer chains results in a strong decrease of the dielectric constant of the polymer chain's environment. Since the columbic attrac-
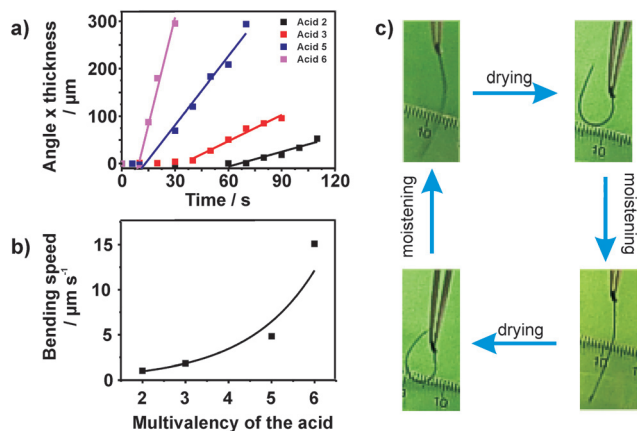

Fig. 1 (a) Photograph of a stripe-shaped membrane. (b) Cross-section view of a porous PIL membrane prepared from PCMVPy-Tf$_2$N with acid **3**. (c) An enlarged view of the same membrane on the top and at the bottom.

Fig. 2 (a) Plot of bending angle × membrane thickness *vs.* time for membrane actuators prepared from different acids. (b) Bending speed derived from the slopes of (b) *vs.* multivalency of the acid. (c) On−off experiment, where a membrane from acid **6** was repeatedly dried and wetted.

tion is indirectly proportional to the dielectric constant of the surrounding medium, the attraction between the polymer and the acid carboxylates increases as water, possessing a relatively large dielectric constant compared to air, is evaporating from the membrane. On account of the gradient in the cross-linking structure of the material, this contraction leads to a membrane bending movement. This explanation was further confirmed by temperature variation. As the dielectric constant of water in its liquid state decreases with increasing temperature,[36] the membranes in water also take a bending conformation when water temperature jumps from 20 to 60 °C.

The difference in the bending speed of the membranes prepared from different acids is related to multiple factors, such as pore size, pore size distribution, pore volume, DEC, DEC distribution, crosslinking density, *etc*. For example, the porous structure compared with the non-porous one is believed to speed up the bending as it facilitates the fast mass-transport of water in and out of the material.[21] This is however only true when the mechanical property of the membrane remains constant or is a secondary effect. The aforementioned factors are often intercorrelated with each other and intrinsically connected to the choice of acid. Therefore, at present it is difficult to dissect the role of each structure factor and to discriminate it from potential subtle changes in others. In spite of this limitation, our data indicate that the choice of acid for the fabrication process is a convenient means to modulate the actuation degree and kinetics.

After analysis of the responsiveness of these membranes in wet and dry environments, their chemical stability in aqueous environment was of intrinsic importance to be examined. The membranes' leaching properties were therefore investigated by taking Fourier-transformed infrared (FTIR)-spectra of the aqueous solution, in which they were stored for months. It was found that neither polymer nor acid compounds were blooming out of the membrane (Fig. S2†), showing their chemical

stability in aqueous environment, although a shrinkage of the average pore size was observed by SEM characterization of the membrane during the stability test.

A common issue in the practical applications of humidity induced actuators is the alteration of their properties by growth of algae, bacteria or fungi. The growth of such organisms may degrade the membrane function and should be desirably inhibited. There are studies about the toxicity of ionic liquids;[37–41] however, little-to-no is reported about their polymeric derivatives, though many synthetic ionic polymers are considered toxic.[42–44] Correspondingly, we performed cytotoxicity tests by MTS assays on two different cell lines, L929 mouse fibroblast cells and CHO hamster ovary cells (Fig. 3a and b). The MTS assay is a standard colorimetric method to determine cell viability by UV-detection of formazan, which is formed from added colorless tetrazolium derivatives by cell enzymes.[45] It was found that the porous membranes strongly inhibited growth of both cell lines. For these studies, we have tested a benzoic acid (**3**), a polymeric acid (**7**), and a natural acid (**9**). From the results, we conclude that the cytotoxicity of these membranes was regardless of the type of acids, as even membranes from natural acids strongly inhibited cell growth. The cationic polymer seems to be the major reason for the membrane cytotoxicity, since it may inhibit cell growth by electrostatic complexation with negatively charged biomolecules, such as proteins or phospholipids.[39–41] The results of the MTS assay show that the tested membranes markedly inhibit the growth of the selected eukaryotic cell lines.

In summary, we have fabricated porous polyelectrolyte membranes by electrostatic cross-linking of a polyvinylpyridinium-based poly(ionic liquid) with multivalent organic acids. The actuation speed and degree when drying the membrane was tuneable in terms of the multivalency of the benzoic acid derivative utilized for electrostatic complexation. Furthermore, we have shown that this material, on account of its charged character, strongly inhibits cell growth and may therefore be used as a potential antifouling coating and material. This work, in general, points out that porous gradient PIL membranes do not need to be necessarily polyvinylimidazolium-based, but are also achievable *via* other PILs, which opens a new dimension to functionalize membranes by structure variation.
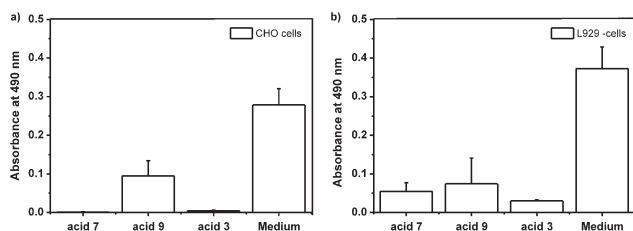

Fig. 3 Results of the MTS cell toxicity tests. (a) CHO cell line. (b) L929 cell line. The absorbance of the cell medium containing *in vivo* generated formazan dye is plotted for membranes from PCMVPy-Tf₂N and different organic acids.

Acknowledgements

Notes and references

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