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Composite hydrogel-loaded alumina membranes for nanofluidic molecular filtration

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ABSTRACT

In this paper a nanofluidic molecular filtration system based on soft alginate hydrogel fillings and a solid-state alumina support membrane is presented. The electrostatically controlled diffusion is characterized by partition coefficient of the hydrogel and the flux through the composite membrane for positively and negatively charged dye molecules. The partition coefficient of negatively charged fluorescein sodium molecules into the gel is 2 orders of magnitude lower in 1 mM KCl solution than that in 1 M KCl solution. The molecular transport properties through the hydrogel loaded alumina membrane are solely dominated by the soft nanoporous hydrogel. Such a composite membrane with alginate hydrogel of only 6 wt% shows a selectivity of 5 for the separation of bovine serum albumin (BSA) and bovine hemoglobin (BHb) in high ionic strength solution of phosphate-buffered saline (PBS).

1. Introduction

Nanoporous membranes are important platforms for various biological and analytical applications such as separation and purification of biomolecules [1–11]. When the pore radius is comparable to the ion screening length known as the Debye length (i.e. length of the electrical double layer), molecular transport through the membrane is electrostatically controlled by the surface charge of the membrane material [12–16]. This property opens a possibility for sorting of biomolecules based on their charges rather than sizes. Nanofluidic filtration systems possess a great potential in separating biomolecules with similar molecular sizes, which is not possible with current commercial membranes.

Aiming at physical nanopaths penetrating through the membrane material, the fabrication of nanoporous membranes is based on advanced micro/nanotechnologies such as controlled electrochemical etching [6,8,12,17,18], track etching [19], focused ion beam [20], e-beam lithography [21], physical vapor deposition [22] and thermal annealing [3]. Recently, nanoporous structures have been achieved in block copolymers membranes via chemical synthesis and self-assembly [7,23]. These structures are in principle solid-state, with possible precision down to a few nanometers [24]. However, the fabrication techniques are expensive and time-consuming, as they rely on specific facilities and complex process flow.

Nanoporous structures can be obtained in soft materials such as polyelectrolyte hydrogels by simple one-step crosslinking [25,26]. For example, alginate sodium salt, a naturally abundant material extracted from brown algae, bears negatively charged chains. The charged polymer chains repel each other during crosslinking, generating sub-100 nm nanoporous network with only a few percent weight of gel polymer (Fig. 1a) [27]. Furthermore, the polymer chains are thermally flexible and mobile at room temperature, resulting in a homogeneous nanoporous system which appears transparent in bulk. Alginate hydrogels are widely used for drug delivery based on the electrostatic interactions between the hydrogels and biomolecules [28–33]. Recently the porous structures of the hydrogels are also developed for tissue engineering [34–37]. The application of hydrogels is generally limited by their poor mechanical stability as they contain very small amount of solid polymer (< 10 wt%).

Here we present a composite molecular filtration system based on soft alginate hydrogel fillings and a solid-state alumina support membrane. Alginate sodium salt solutions of 3, 6 and 9 wt% are mixed with crosslinkers, which can generate alginate hydrogels within a few minutes. The precursor mixture is loaded into a commercial alumina support membrane which is 60 μm thick with holes of 200 nm in diameter, forming a hydrogel/alumina composite membrane (Fig. 1b). The weight percentage of the alginate sodium eventually modulates the overall size of the nanoporous network within the alumina support membrane.

We first characterize the electrostatic exclusion effect of the gel by using negatively charged dye molecules in a microfluidic

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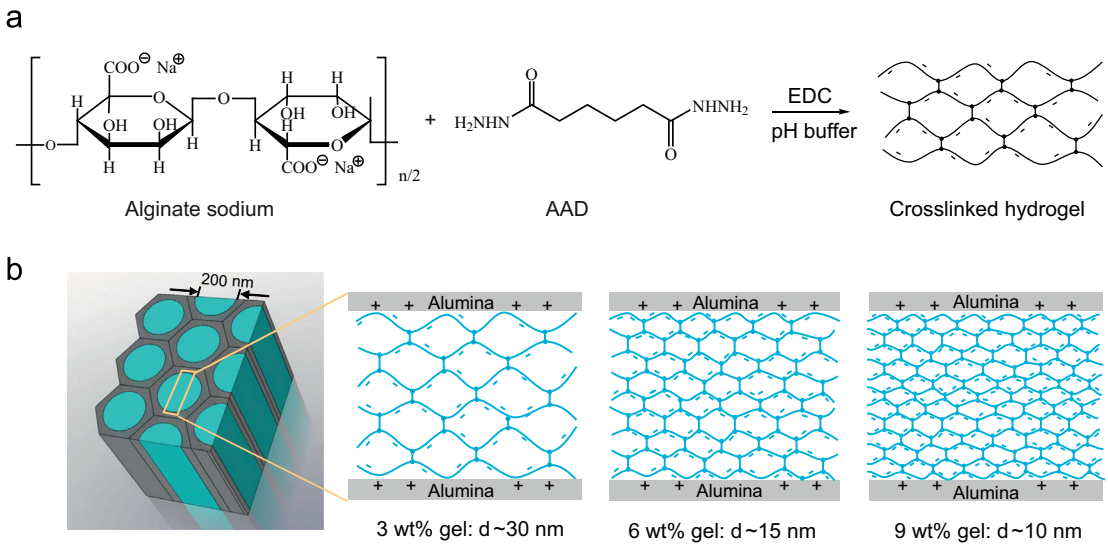

Fig. 1. (a) Crosslinking scheme of alginate hydrogels by using crosslinkers adipic acid dihydrazide (AAD) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC). Negatively charged hydrogel forms a self-organized soft nanoporous network. (b) Schematic of an alginate hydrogel loaded alumina membrane. The size of the nanoporous network can be modulated by the weight percentage of the gel material.

device. Combining the flux of the molecules passing through the composite membrane, the apparent diffusion coefficient through such a nanofluidic membrane can be determined. Finally we demonstrate the potential of such a membrane for separation of similar-sized proteins of bovine serum albumin (BSA) and bovine hemoglobin (BHb).

2. Experimental section

2.1. Materials

All the chemicals were purchased from Sigma-Aldrich without further purification: alginate sodium salt from brown algae (A 0682), adipic acid dihydrazide (AAD, A 0638), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, E 7750), MES hydrate (M 2933), fluorescein sodium (F 6377) and rhodamine 6G (R 4127). The alginate sodium for hydrogel has been characterized concerning its molecular mass. It has a mean molecular weight of 270 kDa [38], which, together with the per residue mass of 200 g/mol ($-C_6H_7O_6Na$), gives a mean polymer length of $z=1350$ residues. Fluorescein labeled alginate sodium molecular probe (MW 150 kDa) was synthesized as previously described [39]. Alumina support membrane was obtained from Whatman (No. 09-926-32), which is 60 μm thick with holes of 200 nm in diameter. Albumin from bovine serum (BSA) labeled with alexa fluor® 488 conjugate was ordered from Life Technologies (A13100). Bovine hemoglobin (BHb) was obtained from Sigma-Aldrich (H 2500).

2.2. Crosslinking of alginate sodium

Long-chained polymers in alginate hydrogels can form networks by either ionic or covalent crosslinks. Ionic crosslinks are formed by the binding of divalent cations e.g., calcium between G blocks on different alginate chains. The ionic bonds, however, are not stable. The crosslinks can dissociate and reform elsewhere in the hydrogel. Especially, the ionic crosslinks can easily break at low ionic strengths. In comparison, covalent crosslinks are much more stable and homogeneous. They can be typically formed by the reaction between carboxylic groups in alginate chains and crosslinking molecules possessing primary diamines. The carboxyl groups are activated by a carbodiimide, EDC, followed by condensation with the amino groups of AAD. The rate of crosslinking (gelation) can be modulated by the pH environment. Gelation rate is slower in solutions with higher pH. For example, the gelation time is 10 s at pH 5.5 and 5 min at pH 5.8. The control of gelation rate is important for the loading of hydrogels into the support membranes. The alginate hydrogel disk samples remain stable in PBS solution at least after 3 months thanks to the robust covalent crosslinks.

The alginate sodium solutions of 30, 60, 90 mg/mL (corresponding to 3, 6 and 9 wt%) were first mixed with AAD and MES pH=5.8 buffer (adjusted with NaOH) at room temperature. The EDC solutions were then added and mixed. The crosslinking/gelation process takes about 5 min. 10% residues react during a complete crosslinking process. The final overall weight percentage of the alginate sodium is given in Table 1.

2.3. Preparation of hydrogel/alumina composite membrane

The alumina membrane was first cleaned in oxygen plasma at 100 W, 0.6 mbar for 3 min. It was then placed on a Buchner funnel which has an open area that fits the size of the membrane. A Venturi pump was connected to provide a constant vacuum (~1 bar) to facilitate the filling of hydrogel. The gel mixture was immediately dropped on the membrane after addition of EDC solutions. The gel slowly filled into the membrane, being crosslinked at the same time. The vacuum was removed after 3 min. The membrane was cleaned and stored in PBS solutions. Excessive gel protruding from the membrane was removed by a scalpel.

2.4. Characterization of partition coefficient

The electrostatic exclusion effect of the alginate hydrogels were characterized by the partition coefficient of negatively charged molecules in a microfluidic device as illustrated in Fig. 2. A drop of hydrogel is confined in a microfluidic channel which is ~50 μm in height. The gel was cleaned and stabilized for 15 h in 1 mM to 1 M KCl solutions before measurement. KCl solutions containing 2 μM fluorescein sodium or fluorescein labeled 150 kDa alginate probe molecule were then introduced into the channel. After stabilizing for another 15 h, the fluorescence intensity was examined under microscope. The partition coefficient K is defined as the ratio of