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# Sustainable Living Filtration Membranes

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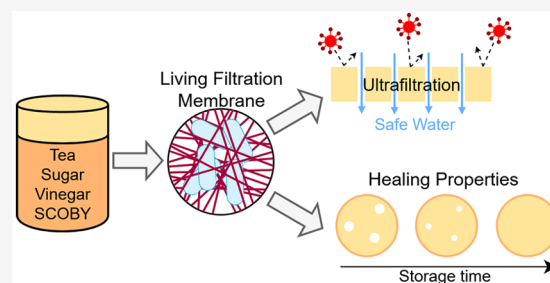


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Supporting Information

**ABSTRACT:** As demand for clean water increases, there is a growing need for effective sustainable water treatment systems. We used the symbiotic culture of bacteria and yeast (SCOBY) that forms while brewing kombucha tea as a living water filtration membrane (LFM). The LFMs function as ultrafiltration membranes with a permeability of  $135 \pm 25 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$  and a 90% rejection of 30 nm nanoparticles. Because they contain living microorganisms that produce cellulose fibers, the surface of an LFM heals after a puncture or incision. Following punctures or incisions, membrane permeability, after a rapid increase postpuncture, returns to 110–250% of the original flux after 10 days in a growth solution. Additionally, LFMs may be manufactured using readily available materials, increasing membrane production accessibility.



## INTRODUCTION

Access to sufficient quantities of clean water is a persistent global problem. In 2015, the United Nations identified providing access to clean water for all as a Sustainable Development Goal,<sup>1</sup> as two-thirds of the world's population (about 3.6 billion people) experience water scarcity for at least 1 month of the year.<sup>2</sup> To combat water scarcity and degrading water quality, we turn to engineered solutions, including advanced water treatment.<sup>3</sup> In water treatment, micro- and ultrafiltration membranes remove pathogens (protozoa like *Giardia* and *Cryptosporidium* and bacteria like *Escherichia coli*) without relying on complex water chemistry required for more conventional methods of water treatment, such as coagulation. Membranes also have a smaller footprint than that of conventional water treatment systems, making them more desirable in urban and decentralized locations. In industry, micro- and ultrafiltration membranes are used to filter and/or concentrate milk,<sup>4</sup> fruit juice,<sup>5</sup> and beer,<sup>6</sup> and they are also used in biomedical applications.<sup>7</sup>

To combat drawbacks associated with traditional polymeric membrane implementation, many have looked to biological systems for inspiration, creating several classes of biomimetic membranes. The incorporation of aquaporins, biological water channels, into synthetic membranes is appealing due to their high water conductivity, which promotes high flux across the membrane.<sup>8,9</sup> However, these biomimetic synthetic membranes are often based on conventional membrane fabrication processes. Unfortunately, conventional polymeric membrane fabrication commonly requires a large amount of harmful solvents, such as the aprotic solvents *N*-methyl-2-pyrrolidone (NMP), *N,N*-dimethylformamide (DMF), and tetrahydrofuran (THF).<sup>10</sup> Workers exposed to DMF, NMP, and THF may

experience hepatic toxicity,<sup>11</sup> developmental toxicity,<sup>12</sup> or neurotoxic and transient hepatic toxic effects, respectively.<sup>13</sup> Thus, membranes that can be manufactured without harmful organic solvents could decrease environmental and health-related impacts.

Some water filtration membranes now incorporate self-healing materials to overcome traditional membrane disadvantages. Self-healing polymers include classes of formaldehydes, epoxies, acrylic acids, and polyelectrolytes, among others.<sup>14–17</sup> While quite promising, traditional self-healing repairs lack permeability in the repaired area, and there is a need for research focused on repeated repair. Meanwhile, biological and cell membranes are able to recover from repeated damage and degradation. Here, we explore potential repeated healing by using living organisms to repair and regenerate a membrane surface.

This paper describes a Living Filtration Membrane (LFM) composed of the bacterial cellulose (BC) network and native microorganisms of a kombucha symbiotic culture of bacteria and yeast (SCOBY). BC has been used in materials research in recent years due to its high tensile strength, biodegradability, and hydrophilicity.<sup>18,19</sup> Some examples of BC applications include “living bandages”, edible food additives, heavy metal adsorbents, and dialysis.<sup>20–23</sup>

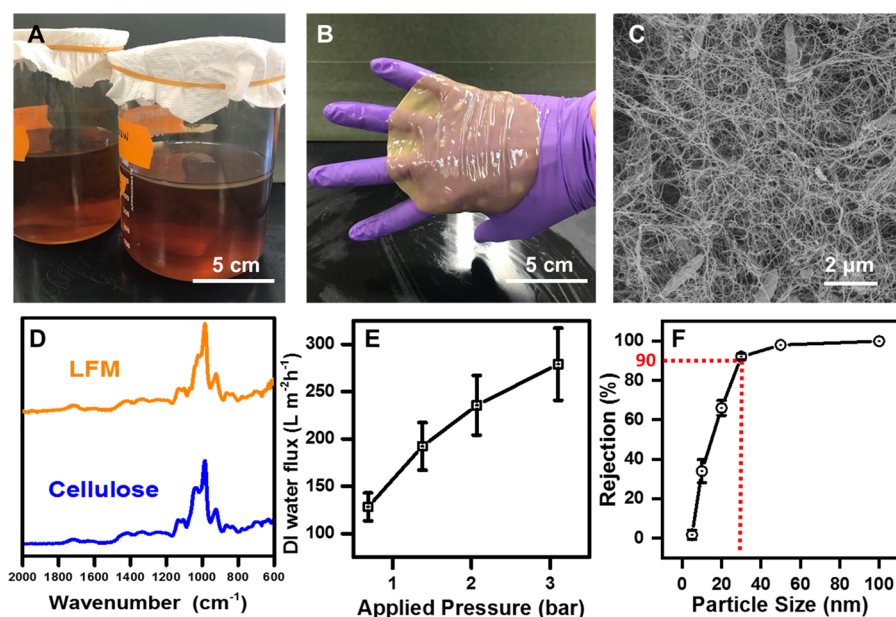
LFMs were fabricated in the laboratory from a mixture of deionized water, black tea, sucrose, acetic acid, and a starter

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**Figure 1.** Living Filtration Membrane (LFM) characteristics: (A) digital photo of an LFM growing at the air–water interface; (B) digital photo of LFM on a gloved hand; (C) scanning electron microscopy (SEM) micrograph of an LFM showing cellulose fibers (sample prepared by dehydration in an isopropanol series and subsequent carbon dioxide critical point drying); (D) Fourier transform infrared (FTIR) spectra of an LFM and a synthetic cellulose fiber mat; (E) dead-end pure water flux as a function of transmembrane pressure for an LFM; (F) LFM selectivity measured with gold and polypropylene nanoparticles. Prior to permeability and selectivity measurements, membranes were compacted at 3.1 bar for 1 h in a dead-end filtration cell. Membrane thickness was  $1.27 \pm 0.20$  mm, and each coupon had a diameter of 25 mm. Where applicable, data are presented as the average with the error bars denoting the standard deviation.

culture of bacteria and yeast (kombucha). The microorganisms feed on the sucrose and form a network of cellulose fibers, which can be used to filter water. The LFMs were characterized for their water filtration, structural, and healing properties. Pristine LFM permeability and nominal pore size were  $135 \pm 25$  L m<sup>-2</sup> h<sup>-1</sup> bar<sup>-1</sup> and 30 nm, respectively. LFM healing tests resulted in a rapid increase in flux followed by a reduction of flux to within 110–250% of the starting flux in a period of 4–17 days, depending on the damage applied. LFMs were tested in the lab as gravity-fed filters, making LFMs a technology that may be useful in places lacking reliable drinking water.

## MATERIALS AND METHODS

**Membrane Growth and LFM Thickness.** A culture of symbiotic bacteria and yeast (Kombucha, 20 g, Cultures for Health) was added to a growth solution consisting of sterile black tea made by boiling 700 mL of deionized water (DI) and steeping 4.6 g of a generic mix of pekoe black teas for 1 h. The growth solution was supplemented with sucrose (85 g, generic, granulated) and distilled white vinegar (200 mL, 5% acetic acid, generic). Mixtures were covered with paper towels, secured with rubber bands, and placed in an incubator at 25 °C for 10 days. After a 10 day growth period, the membranes were stored in an “acidic tea” solution consisting of 4.6 g of generic pekoe black teas and 200 mL of distilled white vinegar. LFMs were used within 2 days.

Prior to any experiments, hydrated LFM thicknesses were quantified by placing a portion of each LFM on a clean microscope slide and measuring the thickness using calipers (United States Plastic Corp, Stainless Steel Caliper) in three different locations to obtain an average thickness. Further details on electrospinning cellulose nanofibers (used as a chemical control), LFM permeability and selectivity testing,

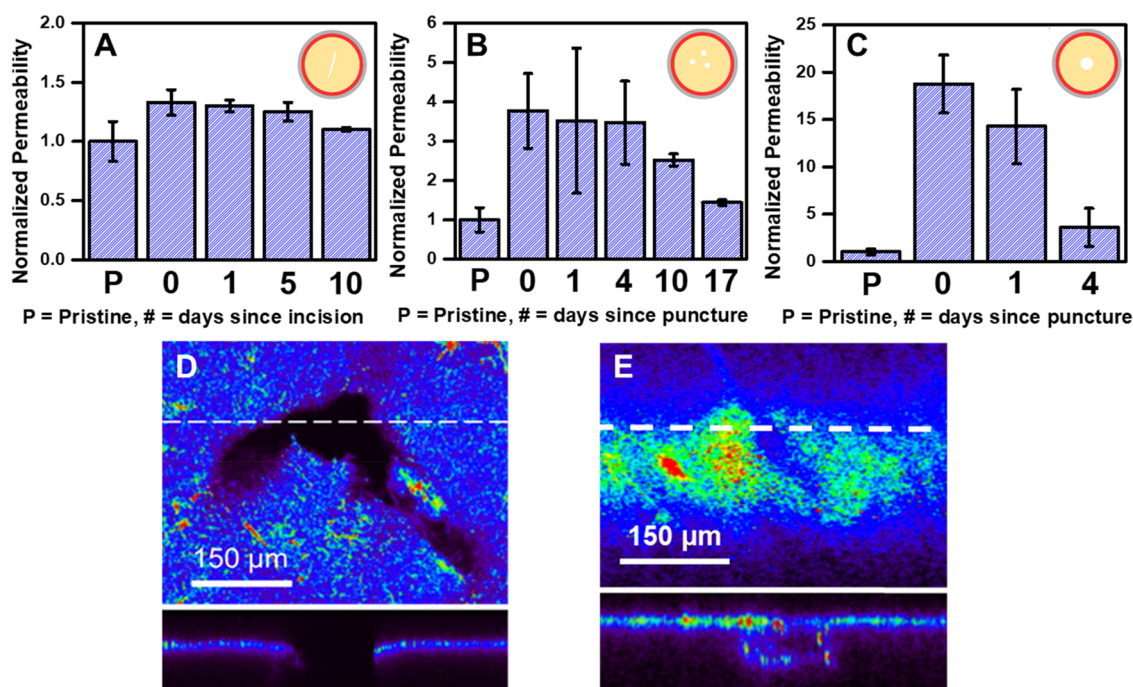
healing tests, and chemical and microscopic membrane characterization are detailed in the [Supporting Information](#).

## RESULTS AND DISCUSSION

**Growing Living Filtration Membranes.** LFMs were grown from ingredients commonly found in grocery stores: dried black tea leaves, distilled white vinegar, sucrose, and a kombucha starter culture. After these ingredients were combined, the mixtures fermented for 7–10 days at 25 °C under nonshaking conditions while a cellulose mat (LFM) grew at the air–water interface (Figure 1A). As inoculation time increases, the LFM thickness increases. We found that 1–1.5 mm thick LFMs showed consistent membrane filtration properties with few defects. Thus, LFMs were harvested from the top of the fermented mixture when they reached a thickness of 1–1.5 mm (Figure 1B). LFMs of this thickness are easily handled in the laboratory in the series of experiments described in this paper without additional support structures or obvious damage.

**LFM Composition and Characteristics.** The morphology of the LFM cellulose matrix was visualized using scanning electron microscopy (SEM, Tescan Mira3, Figure 1C). The LFM was dehydrated for SEM by replacing the water in the LFM with isopropyl alcohol and then replacing the isopropyl alcohol with carbon dioxide via critical point drying (Autosamdri-931 CPD). Then, samples were sputter-coated with gold (Denton-Vacuum, model: Desk-1). The micrograph in Figure 1C reveals fibers with a small average diameter (~40 nm) with entrained microorganisms. The micrographs are consistent with earlier work on bacterial cellulose structures.<sup>23,24</sup>

Cellulose, a long-chain carbohydrate, is the most abundant organic polymer on Earth.<sup>25</sup> It is a building block in plant cell walls and is secreted by some bacteria to form biofilms.<sup>26,27</sup>



**Figure 2.** LFMs grow to fill in incisions and punctures: (A) normalized permeability before and after a 4 mm long surface incision slit, (B) normalized permeability before and after puncture with three 450  $\mu\text{m}$  diameter holes, (C) normalized permeability before and after 3 mm puncture. Insets depict the type of damage. All graphs are normalized to membrane permeability before damage. Membranes were placed in a growth solution to heal for a period of 1–17 days. (D) False-color confocal image of the LFM surface damaged using a 450  $\mu\text{m}$  tapered needle; the bottom of the figure shows a cross-sectional view of the LFM, (E) false-color confocal image of the LFM surface and cross-sectional showing a healing LFM after 14 days.

The presence of cellulose was confirmed using Fourier transform infrared (FTIR) spectroscopy (Nicolet iS5, iD5, with an attenuated total reflectance (ATR) attachment). Spectra (Figure 1D) were collected for the LFM, and a reference synthetic cellulose fiber mat was synthesized by electrospinning<sup>28</sup> (detailed description in the Supporting Information). To produce reference cellulose nanofibers, cellulose acetate was electrospun and converted to cellulose by an alkaline treatment. This formed a random network of nanofibers that had a continuous and cylindrical morphology with an average diameter of  $0.9 \pm 0.5 \mu\text{m}$ .<sup>29</sup> By the absence of a peak at  $1750 \text{ cm}^{-1}$ , the FTIR spectra indicate that the acetate groups of cellulose acetate were replaced with hydroxyl groups and that we successfully fabricated pure cellulose nanofiber mats. Characteristic peaks around  $1020$  and  $1046 \text{ cm}^{-1}$  for both LFM and the cellulose chemical control (Figure 1D) are indicative of C–C, C–OH, C–H ring, and side group vibrations, respectively, confirming that the basis of the LFM is also cellulose.<sup>28</sup>

A static deionized water contact angle (in air) measurement could not be acquired on a wet LFM, which consists of  $\sim 95\%$  water. Furthermore, drying may alter the cellulose fiber structure, potentially changing the contact angle. Thus, the hydrophilicity of the LFMs was determined using captive bubble contact angle measurements. Their contact angle was found to be  $63.1^\circ \pm 5.1^\circ$ , consistent with hydrophilic cellulose-based materials reported in the literature.<sup>29–31</sup>

**Living Filtration Membrane Properties.** LFMs were tested using a 15 mL dead-end filtration cell (Amicon 8101, Millipore Co.) connected to an 800 mL reservoir (Amicon). Pure water permeability was tested after a 1 h compaction period at 3.1 bar. Flux was monitored until stabilization for the

applied pressures of 0.70, 1.4, 2.1, and 3.1 bar. Average permeability was calculated from 18 measurements of 6 different LFMs (thickness  $1.27 \pm 0.204 \text{ mm}$ ). LFM permeability was  $135 \pm 25 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$  (Figure 1E). Depending on applied pressure, traditional polymeric ultrafiltration membrane permeability can be as high as  $\sim 1000 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$ .<sup>32</sup> Therefore, although the permeability of LFMs in this initial study is promising, further work could be performed to increase LFM permeability and decrease variability between samples. LFM selectivity was tested using the same dead-end filtration cell at an applied pressure of 1.38 bar after 1 h compaction at 3.1 bar. Feed solutions consisted of deionized water containing  $100 \text{ mg L}^{-1}$  of particles with known average diameter. Rejection was calculated by measuring the optical density of feed and permeate solutions at 350 and 500 nm for polypropylene and gold nanoparticles, respectively, using UV–vis spectroscopy (Agilent Technologies, Cary 60 UV–vis). The LFM 90% particle diameter cutoff was 30 nm, and LFMs rejected  $>98\%$  of 50 nm and  $>99\%$  of 100 nm particles (Figure 1F). Therefore, LFMs are likely remove most bacteria and protozoa. Traditional polymeric ultrafiltration membranes remove contaminants in the range of 1–30 nm.<sup>33</sup>

**LFMs Grow to Close Incisions and Punctures.** Three healing tests were conducted using 15 or 50 mL dead-end filtration cells (Amicon, Millipore). First, a 4 mm incision was cut into the membrane using a sterile scalpel (width, 0.3 mm) (Figure 2A). Then, three punctures were placed in the membrane with a 450  $\mu\text{m}$  tapered needle (Figure 2B), according to the method of Getachew et al.,<sup>34</sup> who tested self-healing abilities of microcapsule-embedded membranes. Finally, a 2 mm diameter hole was placed in the membrane using a large tapered needle (Figure 2C).

For each damage test, the permeability of the LFM was tested prior to and immediately following damage. Then, the membrane was placed in a growth solution and incubated at 25 °C. After the 4 mm incision, permeability immediately increased to  $130 \pm 15\%$  of the starting permeability and then returned to within  $110 \pm 5\%$  of the intrinsic permeability following 10 days of incubation in a growth solution (Figure 2A). For the second test, permeability increased to  $380 \pm 85\%$  of the starting permeability after puncture with three holes. The permeability returned to  $140 \pm 10\%$  of the starting permeability after 17 days of incubation. For the final test, a large, 3 mm hole was placed in the membrane, causing a permeability increase to  $2000 \pm 280\%$  of the starting permeability. Even after this large puncture, the membrane returned to  $460 \pm 200\%$  of the starting permeability after 4 days. Although the permeability results may be somewhat confounded by changes to the undamaged LFM, the trend suggests that, with appropriate time, the LFM may return to its original flux. It is also likely that the LFM may be able to grow to close a larger defect.

After staining the cellulose fibers with calcoflour white, a confocal microscope (Leica SP8) was used to generate a three-dimensional representation of the LFM surface. Figure 2D shows a rendering of the top of the LFM surface with a visible puncture. The lower cross-sectional view confirms the damage punctured the entirety of the LFM. A similar image of the healed LFM (Figure 2E) indicates a new surface grew over the punctured LFM. This new growth appears to be in a coherent layer that is thinner than the surrounding membrane, indicating the native microorganisms present in the LFM can close openings in the membrane surface.

**Point of Use Applications and Outlook.** Increasing ease of access to clean drinking water is a UN Sustainable Development Goal, and we demonstrate here a method of producing an ultrafiltration membrane that removes suspended particles with size similar to or smaller than bacteria and protozoa. These Living Filtration Membranes (LFMs) are made using materials commonly available at a grocery store or market. Not only can these membranes produce purified drinking water, but also they can be employed using common household items, such as a pour-over coffee maker, without any additional equipment. Figure 3 shows how unsupported LFMs can withstand placement in a coffee filtration device with several centimeters of head. With this setup, 300 mL of water was obtained after 8 h without any additional pressure other than that provided by the deionized water head. Notably, the flow rate could be significantly increased by applying gravity pressure using an elevated water tank and an in-line filter.

Future work in the area of LFMs could focus on increasing the permeability, improving their efficiency, and broadening their applications as ultrafiltration membranes. Although the permeability of these membranes is high for the present stage of technology development, it could be increased by decreasing the thickness of the LFM. Currently, thickness is limited by the method of growth at the liquid–air interface. Our experiments show that a thickness of  $\sim 1.5$  mm yields an LFM with uniform properties over a relatively large area (22 cm). Future work could develop methods to decrease this thickness, potentially through development and use of a support material. Thus, the use of a kombucha SCOBY as an LFM is an interesting development in the area of water treatment membrane fabrication. LFMs can be grown without harmful solvents in



**Figure 3.** Point of use application with potential operational setup for gravity LFM filtration. Inset shows top of filter supporting feedwater.

a low-tech environment, potentially bringing accessible water treatment to anyone, anywhere.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.estlett.0c00019>.

Electrospinning of cellulose nanofibers; permeability and selectivity testing; healing testing; chemical and microscopic characterization (PDF)

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## Notes

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The authors declare no competing financial interest.

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