Chitosan Based Fibrous Absorbents for Indoxyl Sulfate Sorption

Siyu Xiong[*], *Yaxuan Lyu*[**], *Andrew Davenport*[§], *and Kwang Leong Choy*[*†]

* UCL Institute for Materials Discovery, University College London, London WC1E 7JE, United Kingdom.

** MRC Laboratory for Molecular Cell Biology, University College London, London WC1E 6BT, United Kingdom.

§ UCL Centre for Nephrology, Royal Free Hospital, University College London, London NW3 2PF, United Kingdom.

† Corresponding author and e-mail: k.choy@ucl.ac.uk

ABSTRACT

Standard dialyzer membranes, designed for diffusive clearance, do not effectively clear protein-bound uremic toxins, such as indoxyl sulfate (IS). To increase protein-bound toxins removal, absorbents require a high specific surface area to achieve effective size-coupling removal of target toxins. However, the toxicity of a molecule is not necessarily determined by size alone. As proof of concept, we report on an electrospun polycaprolactone/chitosan (PCL/CS) fibrous absorbent for IS removal based on chemical structural interaction. A single unit (20mm in length) of our PCL/CS absorbent achieved a 28% clearance of IS within an hour at both 40mg/L and 5mg/L concentrations in a single pass model. This fibrous absorbent structure offers new thoughts on absorbent design.

KEYWORDS

Fibrous materials; absorbent; ureamic toxin removal; chitosan; electrospinning

1. INTRODUCTION

Indoxyl sulfate (IS) is an protein-bound uremic toxin which accumulates in patients with chronic kidney disease [1]. Standard hemodialyzers, designed for diffusive clearance, do not effectively remove protein-bound uremic toxins, such as IS. There has been an increased interest in the adsorption of uremic toxins, with the development of the wearable artificial kidney (WAK), and recycling of waste dialysate. Most uremic toxin adsorbents have been designed based on "size-coupling" interactions , such as porous carbon [2] and zeolite [3], utilizing pore size to recognize and capture toxins. However, the bio-toxicity of a molecule is not necessarily determined by its size, so that an alternative absorbent, based on interactions related to chemical structures could potentially improve clearances.

Chitosan (CS) is a cost-effective biocompatible electrospunable polysaccharide, which has been used in water filtration systems since the 1990s to remove organic contaminates through interactions between amine groups and conjugated compounds [4]. Different from porous materials, chitosan could potentially interact with the conjugated chemical structure of IS as an effective sorbent manufactured on changeable microfluidic chips [5] for dialysis patients. CS electrospun into fibers provides more effective interactions with the toxin flow because of the higher surface area to volume ratio of the electrospun fibers compared to bulk materials [6]. Additionally, electrospinning can be performed at room temperature, which is beneficial for material function retainment [7]. We chose to combine polycaprolactone (PCL) with CS, due to its stable chemical properties, biocompatibility, and processability by electrospinning [8].

As a proof of concept, PCL and CS were electrospun into a PCL/CS composite fibrous absorbent for IS absorption. We characterized the microstructure and composition of the PCL/CS absorbent,

and tested the absorption performance with IS, to determine the relationship between IS absorption and PCL/CS .

2. MATERIALS AND METHODS

2.1. Materials and fabrication of PCL/CS fibrous absorbents

5.0mL acetone (HPLC grade, Sigma-Aldrich, 34850-2.5L-M) was mixed with 5.0mL formic acid (ACS reagent, Sigma-Aldrich, 33015-1L-M), forming a 50/50 (v/v) solvent mixture. Chitosan (deacetylation = 80.0 ~ 95.0%, SCR, 69047436-100g) powder weighted from 0.04g to 0.20g was mixed with 0.70g polycaprolactone (Mn = 80,000, Sigma-Aldrich, 440744-125G) and dissolved into the solvent mixture, and stirred at 40°C for 24 hours, forming homogenously transparent and slightly yellow viscous precursor solutions for the electrospinning of PCL/CS fibers with different PCL/CS weight ratios (Table S1, Supplementary Data). The PCL/CS fibrous mesh was electrospun from these precursor solutions using a two-dimensional (2D) controlled close-range electrospinning setup, and then rolled and trimmed into a hollow absorbent tubes with a dimension of Φ2.0mm × L20mm (diameter × effective length) and labelled from CS0 to CS20 representing absorbent samples with different PCL/CS weight ratios (e.g. CS0 means no CS in mesh; CS10 means the PCL/CS weight ratio is 70/10). Figure 1 provides an overview of our fabrication process (details in Supplementary data).

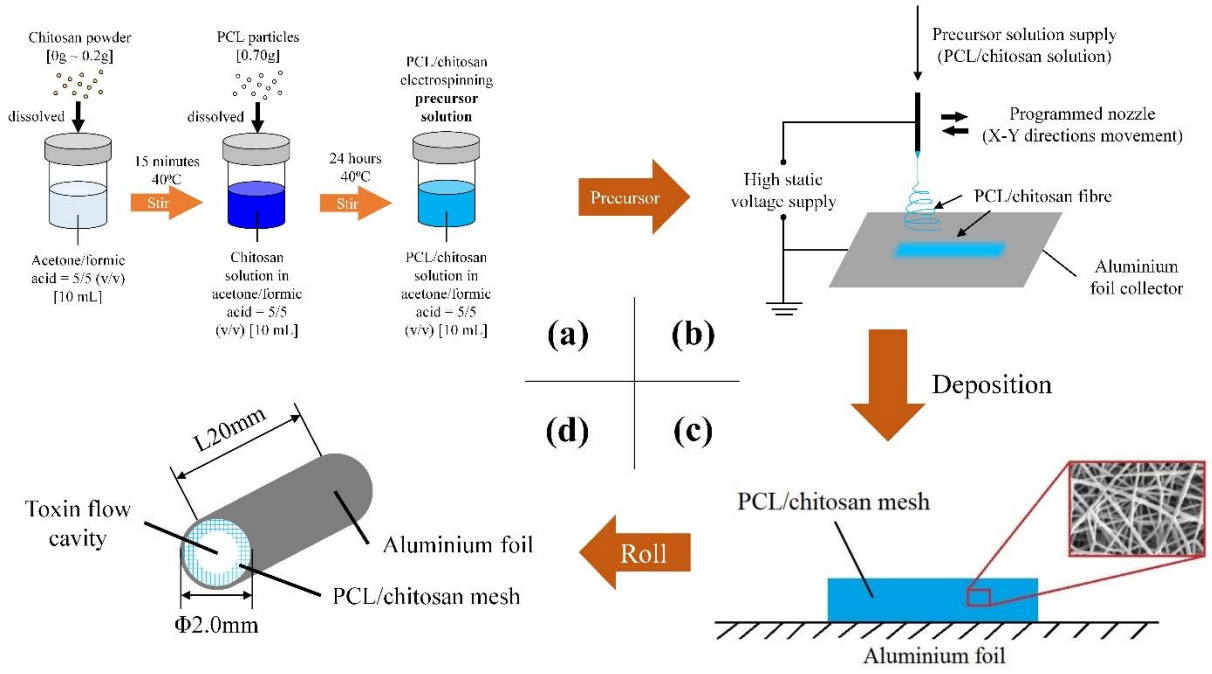

Figure 1. The fabrication steps of the PCL/CS fibrous absorbent. (**a**) The electrospinning precursor solution preparation steps. (**b**) PCL/CS precursor solutions were electrospun into (**c**) fibrous structure, (**d**) subsequently rolled into a hollow tube (Φ for diameter, L for length).

2.2. Absorbent characterization

The PCL/CS absorbents were gold-coated (Q 150R ES, Quorum, UK) before characterization by scanning electron microscopy (SEM) (EVO LS15, Carl Zeiss AG, Germany). The size distribution of fiber diameter and pore width of the PCL/CS meshes were analyzed using ImageJ (ImageJ 1.50i, National Institute of Health, USA) based on randomly selected fibers from 3 sample SEM images of PCL/CS absorbents, and plotted in Origin (Origin Pro 2017, OriginLab Co., USA). The chemical content of the PCL/CS absorbents was characterized using a combination of the Fourier-transform infrared spectroscopy (FTIR) (Spectrum Two, PerkinElmer, Beaconsfield, UK) and the thermogravimetric analyzer (TGA) (TGA 4000, PerkinElmer, UK).

2.3. Absorbent performance evaluation and IS level measurements

The IS absorption performance of the PCL/CS absorbents were evaluated by infusing a solution of IS through the PCL/CS absorbents (Figure S1, Supplementary Data). IS was measured by a high performance liquid chromatography (HPLC) (Flexar, PerkinElmer, USA) equipped with a C18 column (Hypersil, 3µm C18, 130Å, LC Column 250 × 4.6mm, Thermo Scientific, USA) using a UV detector at 278 nm (details in Supplementary data). We compared the initial IS concentration with that in the filtrate after a single pass through the absorbent. We calculated the % IS removal by the PCL/CS absorbent using the following equation:

$$\text{Removed IS percentage } = \frac{C_0 - C_F}{C_0} \times 100\%,$$

where C_F (mg/L) and C_0 (mg/L) were the IS concentration in post filtered solution and the initial IS solution, respectively.

2.4. Statistics

The significance of the IS absorption test results from the PCL/CS absorbents using different PCL/CS weight ratios at a certain initial IS concentration were analyzed and validated using the one-way ANOVA test in Origin (Origin Pro 2017, OriginLab Co., USA). Statistical difference was taken at a p-value of less than 0.05.

3. RESULTS & DISCUSSIONS

3.1. Microstructure of absorbents

The PCL/CS absorbents were recognized as a fibrous mesh with an average 300nm pore width and 170nm fiber diameter (Figure 2; Figure S2, Supplementary Data), which allows IS (Mw = 251Da, molecule diameter = 1.6nm [9]) and human serum albumin (Mw = 61kDa, molecule dimensions = 8.0 × 8.0 × 3.0nm [10]) to readily pass through, while red blood cells (average disc diameter = 7.8µm [11]) could not penetrate. The CS20 (PCL/CS weight ratio = 70/20) was not considered in further tests due to the variance in pore width distribution, indicating poor electrospinning quality of the CS20 mixture. The amine group absorption peak at 1580cm^{-1} wavenumber was observed in the FTIR spectra (Figure 2**c**) for all PCL/CS absorbents as a confirmation of CS content. The degradation points of the PCL/CS meshes were observed at 246°C and 350°C by the TGA analysis (Figure S4, Supplementary Data), validating the PCL and CS content in the absorbent by comparing with the TGA curves of pure CS and PCL, which were degraded at 245°C and 373°C, respectively (Figure S3, Supplementary Data).

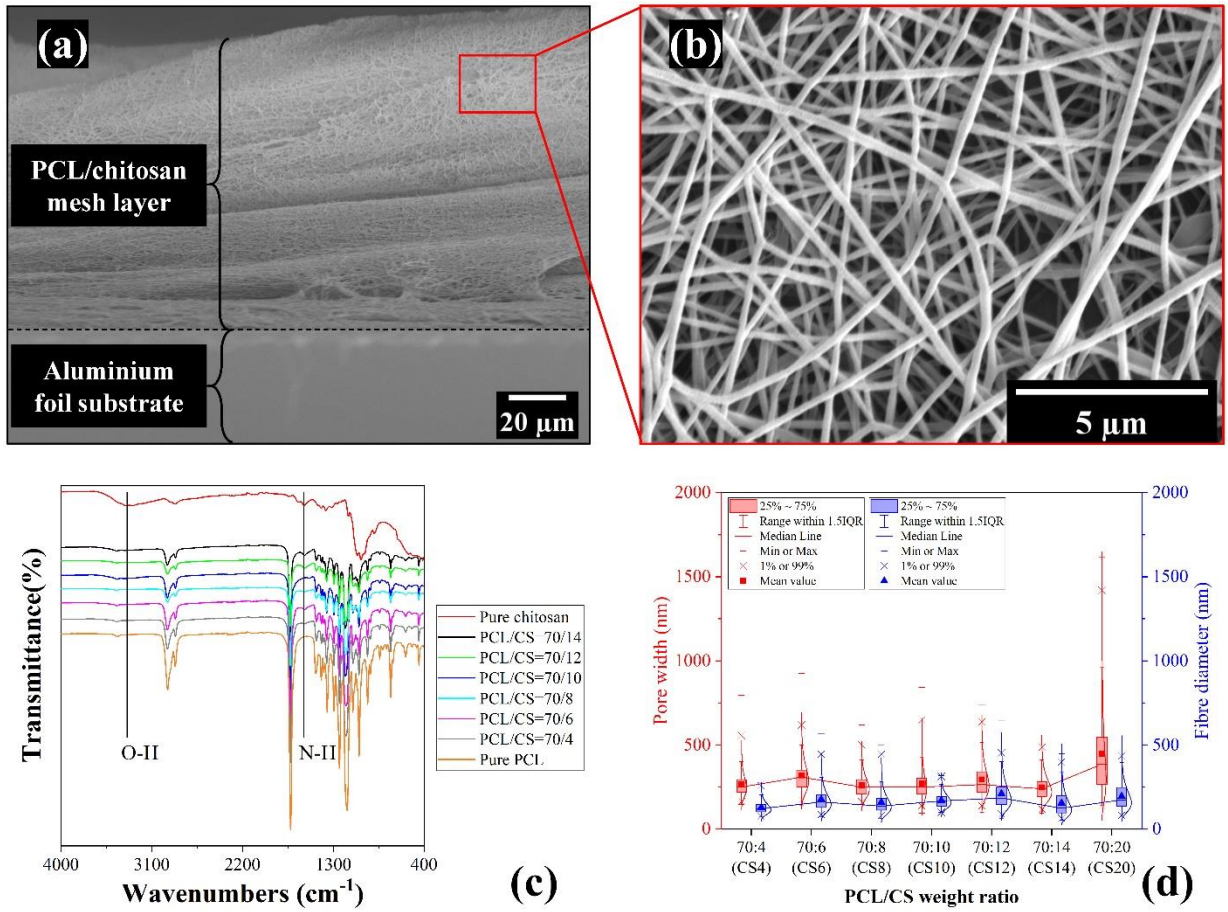

Figure 2. The SEM images of the PCL/CS absorbent (CS10) (PCL/CS weight ratio = 70/10) in (**a**) cross-sectional view and (**b**) surface view. (**c**) The FTIR spectra of the PCL/CS absorbents. (**d**) The size distribution analysis of the fiber diameter and the pore width.

3.2. Evaluation of indoxyl sulfate absorption performance

The PCL/CS absorbents with different PCL/CS weight ratios were evaluated (Figure 3), by comparison using an aluminium foil tube with the same dimensions as the PCL/CS absorbent tube, as a negative control to determine IS absorption. The pure PCL fibers could not absorb IS molecules, which was tested as the CS0 group (Figure 3), proving that the PCL functioned as the supportive content in the PCL/CS composite.

We found that the percentage IS removed was significantly related to the PCL/CS weight ratio from CS0 to CS6. Starting from CS6 to CS14, the percentage IS removed reached a saturation level around 28%, with no significant difference using IS solutions of 5 and 40mg/L (Figure 3), which suggests a non-physical equilibrium regulating the absorption interactions between CS and IS. This interaction was different from the physical adsorption provided by highly porous materials (e.g. porous carbon and zeolite) as a similar percentage IS removal and time course were observed at different starting IS concentrations (Figure 3). The exact mechanism of this absorption interaction has yet to be fully established, but it has been proposed to between the amine groups and other conjugated chemical structures, as CS can absorb conjugated compounds in aqueous solutions, including bisphenol A and quinones [4]. Our results demonstrate the potential application of CS as an absorbent, where both low filtration intensity and material biocompatibility are required, such as miniaturized devices, including the wearable artificial kidney (WAK) and slow continuous ultrafiltration (SCUF) via microfluidics [5].

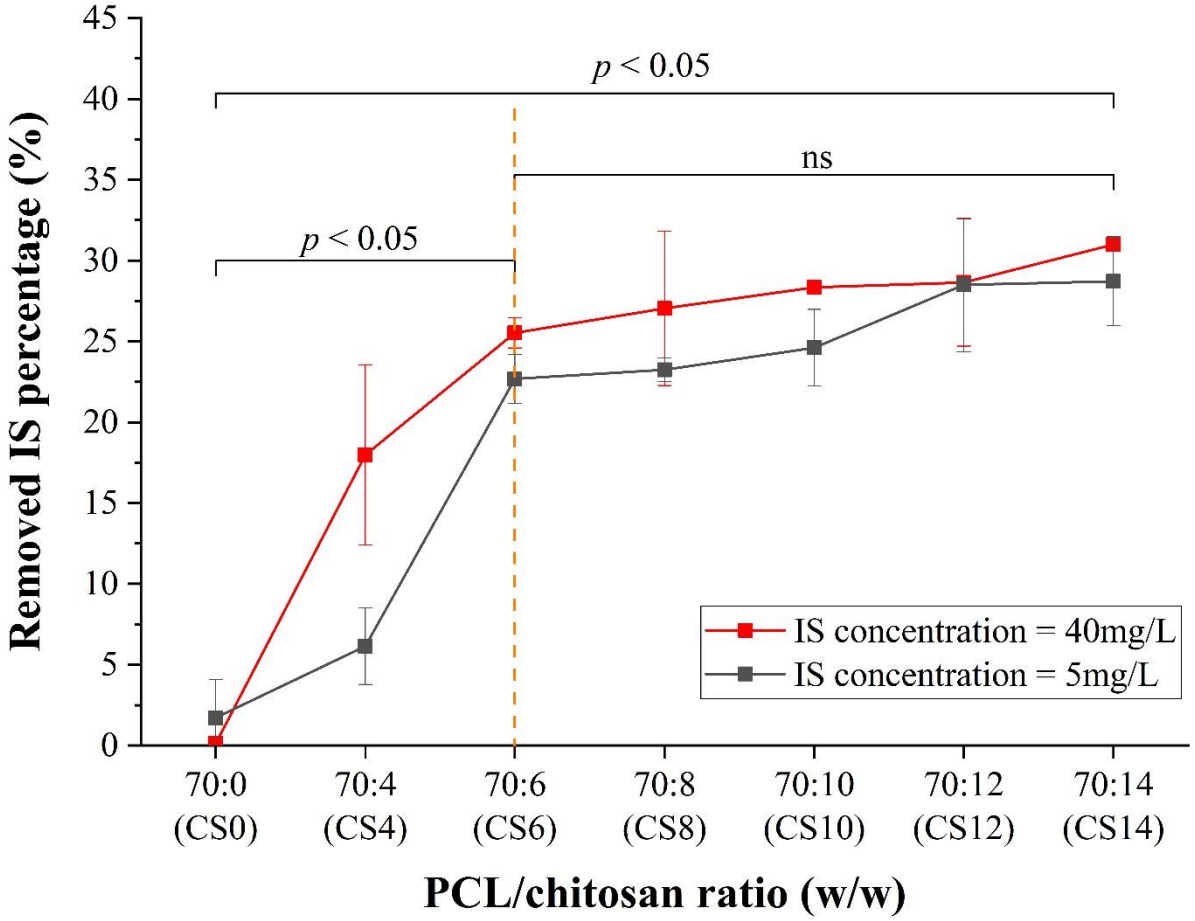

Figure 3. IS absorption performance of the PCL/CS absorbent with different PCL/CS weight ratios at 40mg/L and 5mg/L initial IS concentration . (N = 3, ns = no significant difference)

4. CONCLUSIONS

We have successfully demonstrated that a flexible PCL/CS fibrous absorbent can absorb indoxyl sulfate, as a proof of concept. The absorption of IS at both 5 and 40mg/L was due to non-physical equilibrium between CS and IS with saturation level of 28% after one hour, in a single pass model. The IS absorbability of the PCL/CS absorbent could potentially be optimized in the future by increasing the fiber surface porosity or composing other IS absorptive compounds for filtration applications that require both low filtration intensity and biocompatibility, especially suitable for miniaturized devices, including the wearable artificial kidney (WAK) and slow continuous ultrafiltration (SCUF) via microfluidics. Moreover, this hopefully initiates new thoughts on flexible bio-toxin absorbent design and their viability.

ACKNOWLEDGEMENT

We would like to thank Oliver Walker for his support in using TGA and HPLC equipment. The partial support of Therapeutic Acceleration Support (TAS) Fund and St. Peter's Trust Fund are gratefully acknowledged.

Author Contributions

Siyu Xiong: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft; **Yaxuan Lyu:** Methodology, Visualization; **Andrew Davenport:** Funding acquisition, Supervision, Writing – review & editing; **Kwang Leong Choy:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

1. Meijers, B.K., Bammens, B., Verbeke, K., and Evenepoel, P., *A review of albumin binding in CKD.* Am J Kidney Dis, 2008. **51**(5): p. 839-850.

2. Pavlenko, D., Giasafaki, D., Charalambopoulou, G., van Geffen, E., Gerritsen, K.G.F., Steriotis, T., and Stamatialis, D., *Carbon Adsorbents With Dual Porosity for Efficient Removal of Uremic Toxins and Cytokines from Human Plasma.* Sci Rep, 2017. **7**(1): p. 14914.

3. Lu, L., Samarasekera, C., and Yeow, J.T.W., *Creatinine adsorption capacity of electrospun polyacrylonitrile (PAN)-zeolite nanofiber membranes for potential artificial kidney applications.* Journal of Applied Polymer Science, 2015. **132**(34).

4. Yong, S.K., Shrivastava, M., Srivastava, P., Kunhikrishnan, A., and Bolan, N., *Environmental applications of chitosan and its derivatives.* Rev Environ Contam Toxicol, 2015. **233**: p. 1-43.

5. Lee, K.H., Kim, D.J., Min, B.G., and Lee, S.H., *Polymeric nanofiber web-based artificial renal microfluidic chip.* Biomedical Microdevices, 2007. **9**(4): p. 435-442.

6. Bellan, L.M. and Craighead, H.G., *Nanomanufacturing Using Electrospinning.* Journal of Manufacturing Science and Engineering, 2009. **131**(3).

7. Mendes, A.C., Stephansen, K., and Chronakis, I.S., *Electrospinning of food proteins and polysaccharides.* Food Hydrocolloids, 2017. **68**: p. 53-68.

8. Kim, Y.-J., Park, M.R., Kim, M.S., and Kwon, O.H., *Polyphenol-loaded polycaprolactone nanofibers for effective growth inhibition of human cancer cells.* Materials Chemistry and Physics, 2012. **133**(2-3): p. 674-680.

9. Wernert, V., Schäf, O., Ghobarkar, H., and Denoyel, R., *Adsorption properties of zeolites for artificial kidney applications.* Microporous and Mesoporous Materials, 2005. **83**(1-3): p. 101-113.

10. Sugio, S., Kashima, A., Mochizuki, S., Noda, M., and Kobayashi, K., *Crystal structure of human serum albumin at 2.5 Å resolution.* Protein engineering, 1999. **12**(6): p. 439-446.

11. Cetin, A. and Sahin, M., *A monolithic fluid-structure interaction framework applied to red blood cells.* Int J Numer Method Biomed Eng, 2019. **35**(2): p. e3171.